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Clinical Evaluation of 6-Mercaptopurine in the Treatment of Leukemia. By JOSEPH H. BURCHENAL, M.D., DAVID A. KARNOFSKY, M.D., M. LOIS MURPHY, M.D., ROSE RUTH ELLISON, M.D., MARGUERITE P. SYKES, M.D., CHARLOTTE T. C. TAN, M.D., ALAN C. MEMRANN, M.D., MUNIRE YUCEOGLU, M.D., and C. P. RHOADS, M.D. 371

The Fanconi Syndrome and Its Clinical Variants. By RALPH M. MYERSON, M.D., and BERNARD H. PASTOR, M.D. 378

Myeloid Metaplasia as a Sequela of Polycythemia. By EDWARD STEINFIELD, M.D., and LAWRENCE HARVEY BEIZER, M.D. 388

Studies on the Control of Hypertension by Hyphex. III. Pharmacological and Chemical Observations on 1-Hydrazinophthalazine. By H. MITCHELL PERRY, JR., M.D., and HENRY A. SCHROEDER, M.D. 396

Studies on the Control of Hypertension by Hyphex. IV. Levels of the Agents in Urine and Blood. By H. MITCHELL PERRY, JR., M.D., HENRY A. SCHROEDER, M.D., and JOHN D. MORROW, M.D. 405

The Gastroesophageal Vestibule, Its Normal Function and Its Role in Cardiospasm and Gastroesophageal Reflux. By FRANZ J. INGELFINGER, M.D., PHILIP KRAMER, M.D., and GUILLERMO C. SANCHEZ, M.D. 417

The Effect of Proteolytic Enzymes on the Physical and Chemical Characteristics of the Tracheobronchial Secretions of Patients with Poliomyelitis. By SIDNEY KOFMAN, M.D., MARK H. LEPPER, M.D., GEORGE GEE JACKSON, M.D., and HARRY F. DOWLING, M.D. 426

The Use of Trypsin or Streptokinase-Streptodornase for the Therapy and Prevention of Atelectasis. By SIDNEY KOFMAN, M.D., MARK H. LEPPER, M.D., GEORGE GEE JACKSON, M.D., and HARRY F. DOWLING, M.D. 432

Diuretic Effect and Local Tolerance of a New Type of Mercurial Rectal Suppository: A Preliminary Report. By JAMES W. DALY, M.D. 440

Complement Components in the Sera and Urines of Patients with Severe Proteinurias. By KURT LANGE, M.D., and EUGENE J. WENK, M.S. 448

Investigations into the Site of Complement Loss in Experimental Glomerulonephritis. By KURT LANGE, M.D., and EUGENE J. WENK, M.S. 454

On the Forssman-like Behavior of Cold Hemagglutinins for Sheep Erythrocytes. By CHRIS J. D. ZARAFONETIS, M.D., and VIRGINIA F. COLVILLE, B.S. 461

Progress of Medical Science

Oto-Rhino-Laryngology—Recent Trends in Nasal Medication. By NOAH D. FABRICANT, M.D. 465

Dermatology and Syphilology—Nodular Vasculitis. By HERMAN BEERMAN, M.D., and GEORGE H. MITCHELL, M.D. 469

Book Reviews and Notices 487

Complete contents on adv. page 10

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*Blood Pressure, Report of Joint Committee on Mortality of The Association of Life Insurance Medical Directors and The Actuarial Society of America, New York, 1925, pp. 10, 52.

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CONTENTS

ORIGINAL ARTICLES

Clinical Evaluation of 6-Mercaptopurine in the Treatment of Leukemia 371
By Joseph H. Burchenal, M.D., David A. Karnofsky, M.D., M. Lois Murphy, M.D., Rose Ruth Ellison, M.D., Marguerite P. Sykes, M.D., Charlotte T. C. Tan, M.D., Alan C. Mermann, M.D., Munire Yuceoglu, M.D., and C. P. Rhoads, M.D., New York, New York.

The Fanconi Syndrome and Its Clinical Variants 378
By Ralph M. Myerson, M.D., and Bernard H. Pastor, M.D., Philadelphia, Pennsylvania.

Myeloid Metaplasia as a Sequela of Polycythemia 388
By Edward Steinfield, M.D., and Lawrence Harvey Beizer, M.D., Philadelphia, Pennsylvania.

Studies on the Control of Hypertension by Hyphex. III. Pharmacological and Chemical Observations on 1-Hydrazinophthalazine 396
By H. Mitchell Perry, Jr., M.D., and Henry A. Schroeder, M.D., St. Louis, Missouri.

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By H. Mitchell Perry, Jr., M.D., and Henry A. Schroeder, M.D., St. Louis, Missouri.

The Gastroesophageal Vestibule, Its Normal Function and Its Role in Cardiospasm and Gastroesophageal Reflux 417
By Franz J. Ingelfinger, M.D., Philip Kramer, M.D., and Guillermo C. Sanchez, M.D., Boston, Massachusetts.

The Effect of Proteolytic Enzymes on the Physical and Chemical Characteristics of the Tracheobronchial Secretions of Patients with Poliomyelitis 426
By Sidney Kofman, M.D., Mark H. Lepper, M.D., George Gee Jackson, M.D., and Harry F. Dowling, M.D., Chicago, Illinois.

The Use of Trypsin or Streptokinase-Streptodornase for the Therapy and Prevention of Atelectasis 432
By Sidney Kofman, M.D., Mark H. Lepper, M.D., George Gee Jackson, M.D., and Harry F. Dowling, M.D., Chicago, Illinois.

Diuretic Effect and Local Tolerance of a New Type of Mercurial Rectal Suppository: A Preliminary Report 440
By John W. Daly, M.D., Philadelphia, Pennsylvania.

Complement Components in the Sera and Urines of Patients with Severe Proteinurias 448
By Kurt Lange, M.D., and Eugene J. Wenk, M.S., New York, New York.

Investigations into the Site of Complement Loss in Experimental Glomerulonephritis 454
By Kurt Lange, M.D., and Eugene J Wenk, M.S., New York, New York.

On the Forssman-like Behavior of Cold Hemagglutinins for Sheep Erythrocytes 461
By Chris J. D. Zarafonetis, M.D., and Virginia F. Colville, B.S., Philadelphia, Pennsylvania.

(Contents continued on page 12)

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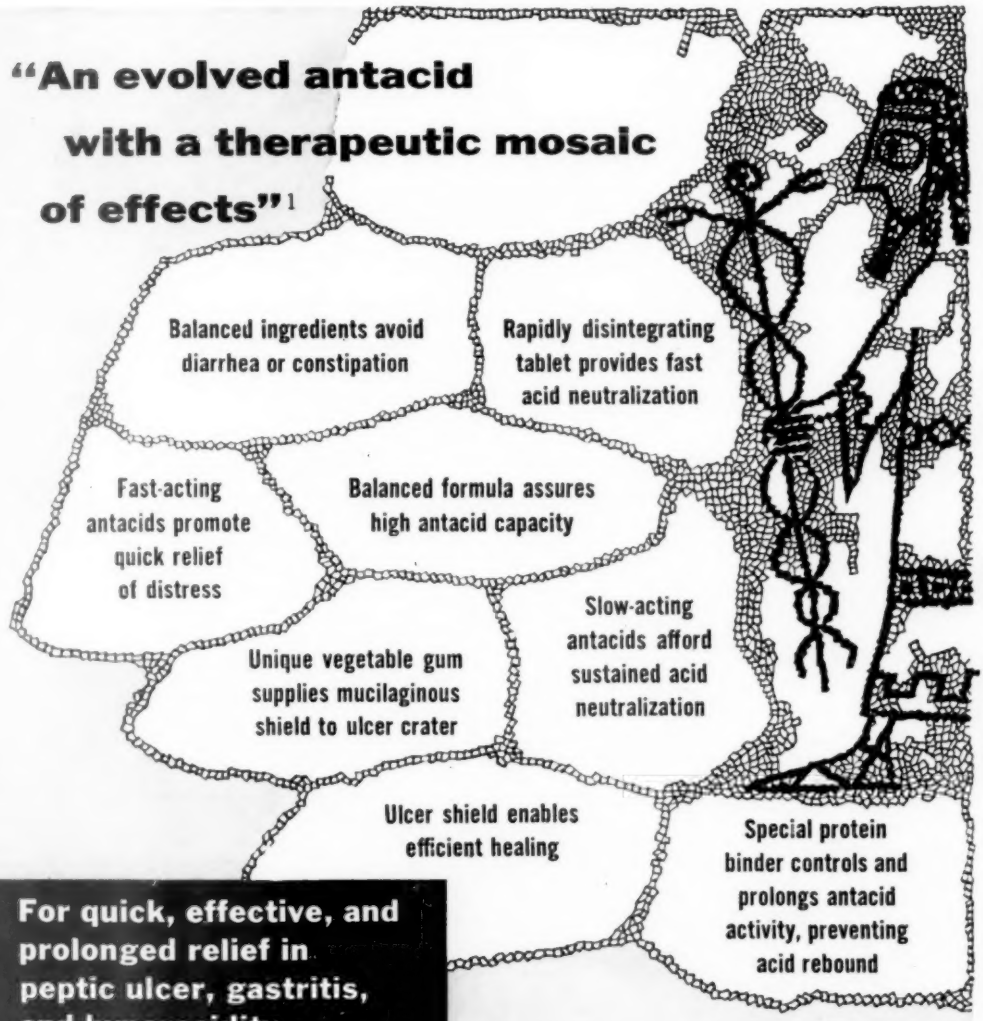
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(*Contents continued on page 14*)

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OCTOBER, 1954

ORIGINAL ARTICLES

CLINICAL EVALUATION OF 6-MERCAPTOPURINE IN THE TREATMENT OF LEUKEMIA*

By Joseph H. Burchenal, M.D.
David A. Karnofsky, M.D.
M. Lois Murphy, M.D.
Rose Ruth Ellison, M.D.
Marguerite P. Sykes, M.D.
Charlotte T. C. Tan, M.D.
Alan C. Mermann, M.D.
Munire Yuceoglu, M.D.
AND
C. P. Rhoads, M.D.

(From the Chemotherapy Service, Memorial Center for Cancer and Allied Diseases, Division of Experimental Chemotherapy, Sloan-Kettering Institute, and the Sloan-Kettering Division of Cornell University Medical College, New York, New York)

Presented at the Association of American Physicians, May 5, 1954, Atlantic City, New Jersey.

The synthesis of 6-mercaptopurine by Elion, Hitchings, *et al.*[7] was the result of a long-term cooperative project between the Wellcome Research Laboratories and the Sloan-Kettering Institute to study, for their antitumor and antileukemic effects, analogs of precursors of nucleic acid. The tumor-inhibiting activity of this compound was first demonstrated by Clarke *et al.*[4] against Sarcoma 180 in mice.

In *Lactobacillus casei* 6-mercaptopurine acts as a purine antagonist[8] and can be reversed by adenine, guanine, xanthine, or hypoxanthine, but in mammals it has been impossible to reverse either the toxic, the antitumor or the antileukemic effects of mercaptopurine by any of these simple purines[2,5]. Both Law[11] and Goldin[9], however, have reported some success in reversing the antileukemic activity of this compound in mice by adenylic acid.

Elion has demonstrated the incorporation of the radio-activity of 8-C^{14} and S^{35} labelled 6-mercaptopurine into

*This investigation was supported by a research grant from the National Cancer Institute of the National Institutes of Health, Public Health Service; institutional grant from the American Cancer Society, the Damon Runyon Memorial Fund for Cancer Research, Lasker Foundation, Grant Foundation, and the Black-Stevenson Foundation.

the nucleic acids of both RNA and DNA fractions of the total viscera of normal mice[6]. Hamilton and Elion, in studying the fate of 6-mercaptopurine in man after an intravenous dose of S^{35} labelled compound, noted incorporation of radioactivity into the DNA of the cells of chronic myelocytic leukemia[10].

The therapeutic activity of this compound has been evaluated by the Chemotherapy Service, Memorial Center, in 269 patients with neoplastic disease, of whom 140 had acute leukemia, 8 subacute, 18 chronic myelocytic, and 4 chronic lymphocytic leukemia. The usual dose in children was 2.5 mg./kg. by mouth in a single daily dose and at this level very few toxic manifestations were observed. This dosage is usually tolerated by adults, but in some patients evidence of bone marrow depression has occurred. At higher levels severe depression of all formed elements of the marrow was occasionally a serious hazard, but in some patients, both adults and children, 5 to 7 mg./kg. were tolerated without difficulty. At these doses mild gastrointestinal disturbances sometimes occurred. At 2.5 mg./kg. daily the antileukemic effect of mercaptopurine generally did not become manifest until after 3 to 8 weeks of continuous therapy although occasionally it acted more rapidly[1,3].

Mercaptopurine had no practical value in chronic lymphocytic leukemia, lymphosarcoma, Hodgkin's disease or any of the metastatic carcinomas so far studied. In patients with metastatic reticulum cell sarcoma slight temporary regressions of tumor masses and subjective improvement were occasionally noted.

In 11 out of 12 patients in the early stage of chronic myelocytic leukemia, it was effective in producing satisfactory remissions with definite fall in white count, decrease in immature forms, rise in hemoglobin, and a decrease in splenomegaly and hepatomegaly.

Figure 1 demonstrates this fall in total leukocytes, decrease in immature forms, and decrease in spleen size in a patient with chronic myelocytic leukemia. As can be seen in this case and as was noted in most of the other patients, a rapid relapse in approximately 4 weeks occurred when the drug was discontinued. For this reason it is advisable to give maintenance therapy in almost all cases. Occasionally, however, as shown in Fig. 2, therapy continued over a 3- or 4-month period may eventually bring the count down to normal levels and the discontinuation of therapy may then not be followed immediately by an increase in leukocyte count. This patient has been carried for 11 months now without further therapy. How mercaptopurine will compare with the more conventional agents such as Roentgen ray and P^{32} or urethane in the treatment of the early stages of chronic myelocytic leukemia cannot be stated at this time. Patients whose disease has progressed to the terminal acute stage with a high percentage of blasts in the marrow and peripheral blood and with a fall in platelets and hemoglobin rarely respond to the conventional therapeutic agents. It is of considerable interest that 2 out of 6 patients in this stage of the disease have also responded to mercaptopurine with temporary remissions lasting 1 to 3 months, with a decrease in blast forms in marrow and peripheral blood, an increase in hemoglobin and platelets, and clinical improvement.

Of 87 children with acute leukemia, 41 had good clinical and hematologic remissions, 16 had partial remissions and 30 were considered failures. By a good clinical and hematologic remission is meant a marrow in which the total of leukemic cells and lymphocytes

decreased to a level of less than 30%, and in which normal erythroid and myeloid elements increased to at least 70%, megakaryocytes reappeared, the peripheral blood values became essentially normal, and the patient returned temporarily to good health.

Figure 3 shows the response of a 6-year-old child to mercaptopurine therapy with a diminution of leukemic cells and lymphocytes in the marrow, and a rise in platelets and in the percentage of polymorphonuclear leukocytes to normal levels. This remission lasted 6 months, but then despite continuation of therapy there was a relapse. Such remissions lasted in other patients from 1 to 10 months. At this stage, however, this patient was still responsive to amethopterin. In a series

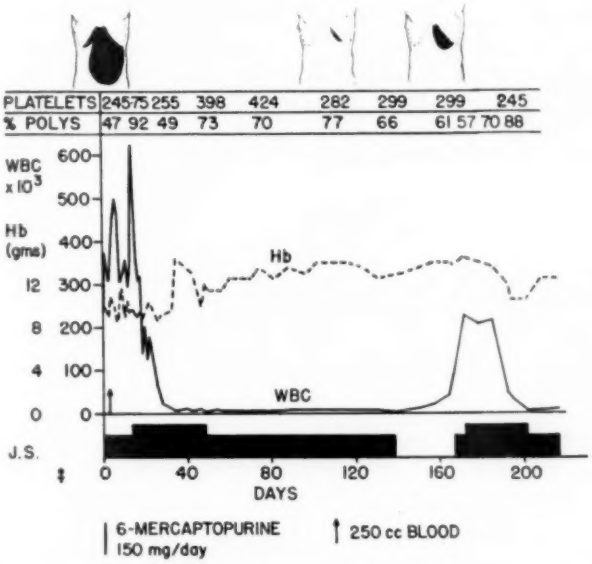

Fig. 1.—Patient with chronic myelocytic leukemia, showing fall in total leukocytes, rise in hemoglobin, and decrease in spleen size under treatment with mercaptopurine.

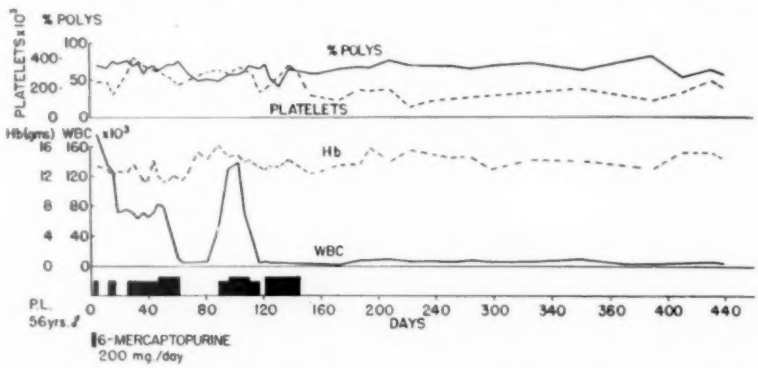

Fig. 2.—Prolonged remission in chronic myelocytic leukemia following mercaptopurine.

of 37 children whose disease had become resistant to 6-mercaptopurine, 14 had good clinical and hematological remissions with amethopterin. Conversely many patients who were initially resistant or who had become resistant to amethopterin or cortisone still responded to mercaptopurine.

Among the 50 adults with acute leukemia the results were not so satisfactory, with 7 good clinical and hematologic remissions, 10 clinical but only partial hematological remissions, and 33 failures. Figure 4 shows a good remission in a 52-year-old woman with acute monocytic leukemia. It can be seen that, under therapy the marrow gradually returned to normal morphology and to a normally functioning state as shown by the fact that the polymorphonuclear leukocytes, hemoglobin and platelets were maintained at normal levels. The patient remained in remission and was able to continue a normal life for over a year under constant maintenance therapy, but is now beginning to show a bone marrow relapse despite continuing therapy. Figure 5 shows what we consider to be a partial remission in a 34-year-old man. Although the leukemic cells and lymphocytes in this patient's marrow never reached a level of below 40%, his marrow was normally functional, the hemoglobin, the platelets, and the percentage of polymorphonuclear leukocytes in the peripheral blood returned to normal levels, and the patient appeared to be in perfect health during a period of 9 months under therapy with the drug. Eventually, however, his disease became resistant to therapy.

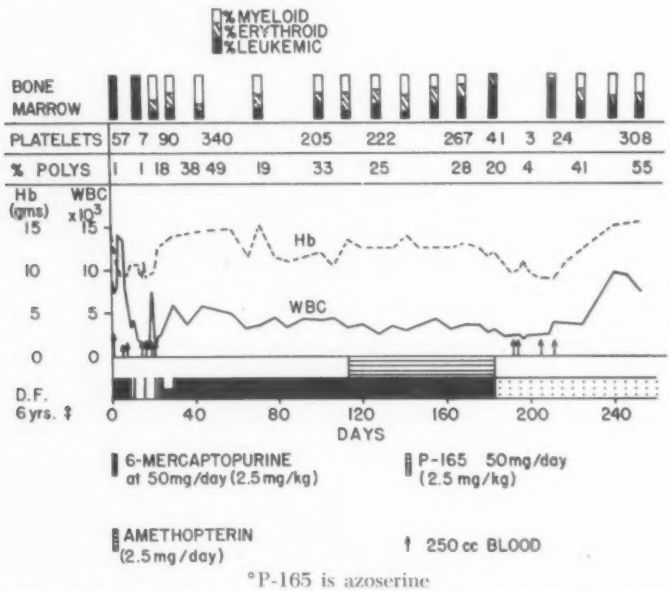

Fig. 3.—Response of a 6-year-old child with acute leukemia to mercaptopurine and later to amethopterin.

In isolated cases in adults, it would appear that the survival time has been increased, but the percentage in which such benefit occurs is so small that it is not possible to demonstrate a statistically valid increase in over-all survival time of adult patients with this disease.

In the treatment of acute leukemia in children we now have at our disposal three types of agents: the folic acid antagonists, the purine antagonists, and the hormones. Fortunately, there is no cross resistance between the different groups and a patient whose disease has become resistant to mercaptopurine may still be expected to respond to amethopterin or cortisone and *vice versa*. All of these drugs are useful, but differences in the rapidity of their action, the duration of the remissions induced, the height of the leukocyte count and the age of the patient suggest the use of a particular agent in a given situation. In any patient with acute leukemia who is acutely ill and has a marked hemorrhagic diathesis, it is imperative to use a drug which acts rapidly, and therefore cortisone or ACTH would be the drugs of choice, except possibly in monocytic or myelomonocytoid leukemia. On the other hand, in

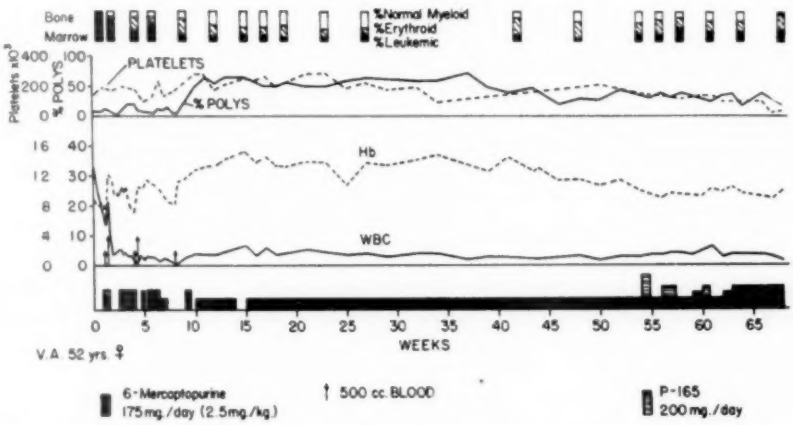

Fig. 4.—Effect of 6-mercaptopurine in a patient with acute monocytic leukemia. (P-165 is azoserine)

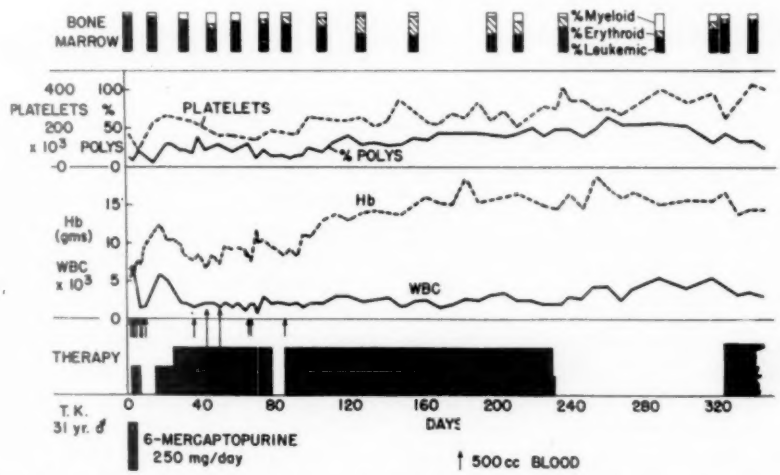

Fig. 5.—Partial hematological but good clinical remission following mercaptopurine.

the patient with acute leukemia who has minimal hemorrhagic manifestations and who appears likely to survive the 3 to 8 weeks necessary to bring about a good remission with the folic acid or purine antagonists, the use of these agents is preferable as longer and more satisfactory remissions may be expected. In adult patients or in those children whose total leukocyte count is initially over 50,000, mercaptopurine seems to be somewhat safer and of greater value than amethopterin, but in children with leukocyte counts under 50,000 the remissions caused by the folic acid antagonists may be somewhat longer.

Thus it is our feeling that in acute leukemias, the antimetabolites (amethopterin and mercaptopurine) should be the main reliance of the chemotherapist, with the hormones (cortisone and ACTH) being reserved for the emergency situations, where the disease has become resistant to the antimetabolites or where there is not sufficient time for the use of these slower-acting drugs.

Although combination therapy theoretically appears to be the most promising method for controlling leukemia, combinations of amethopterin, cortisone and mercaptopurine have not in our experience been any more satisfactory than the sequential use of these drugs. By using the various drugs available in the sequence indicated above, a definite increase in survival time can be demonstrated. Table 1 shows the survival time of 3 different groups of children with acute leukemia. In 218 untreated cases collected from the literature by Tivey[12], 50% were dead by 3.9 months and only 5% were alive 12 months after the start of their disease. In the second series of 154 children treated at Memorial Hospital with the folic acid antagonists or the steroids, or both, 50% were dead by 8.9 months and 29% were living 12 months after the start of their disease. In the third group of 52 cases, in which treatment was started during the period June 1, 1952, to April 30, 1953, at the time when mercaptopurine became available in addition to the antifolics and steroids, 50% were dead by just over 12 months and the 12-month survival was 52%. Since the latter two series had equal opportunities for antibiotics and transfusions, it would seem that the addition of mercaptopurine to a regimen employing the other two types of agents has caused a definite increase in the survival time of these children.

TABLE 1.—RESULTS OF SEQUENTIAL THERAPY OF ACUTE LEUKEMIA IN CHILDREN

	Number of Patients	*Surviving 1 Year or Longer*
No treatment	218	5%
Cortisone, Amethopterin	154	29%
Amethopterin, Cortisone, and 6-Mercaptopurine	52	52%

Summary. We have in mercaptopurine a new type of agent which has demonstrated activity against experimental tumors and leukemias in the mouse and against leukemia in man. As shown by cross resistance studies, it acts through mechanisms differing from those of the steroids or the folic acid antagonists. A high percentage of temporary remissions can be achieved with this drug in children with acute leukemia. Occasional remissions also occur in adults but the results are generally not as satisfactory as with the children. In early chronic myelocytic leukemia, excellent remissions can be achieved, but here as in the acute leukemias, maintenance therapy must generally be continued. Occasional beneficial effects have been seen in the

terminal acute stage of chronic myelocytic leukemia, but these remissions have been of short duration. Mercaptopurine thus adds another type of antileukemic agent to the armamentarium of the clinician.

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THE FANCONI SYNDROME AND ITS CLINICAL VARIANTS*

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During the past few years there has been renewed interest in a syndrome characterized by renal glycosuria, aminoaciduria, low serum inorganic phosphorus and osteomalacia. Credit for the original description of this syndrome is usually given to Fanconi[27,28] and de Toni[57] and the condition is commonly associated with their names. Cases with some of its features had, however, previously been reported by Schier and Stern[50] in 1926, and Boyd[8] in 1927.

The etiology of the Fanconi syndrome is not known. A hereditary factor seems certain in view of the preponderance of cases in infants and children and the marked familial incidence. The occurrence of the syndrome in adults suggests the possibility of a prolonged latent period for its development or that there is an acquired type of disorder. The latter possibility is favored by the fact that in only 2 of the cases reported in adults has there been a familial history[41,55].

Since Fanconi described the syndrome in 1931, studies have shown fairly conclusively that the underlying pathology is in the kidney and consists of a defect in the function of the proximal convoluted tubules leading to faulty reabsorption of glucose, amino acids, phosphate and occasionally water, bicarbonate and potassium. There is no associated defect in glomerular function and renal failure of the usual clinical variety does not occur.

The relatively few cases that have come to necropsy have all revealed degenerative changes in the proximal tubules of the kidney[1,15,27,44,55]. Two groups of investigators have reported the absence of intracellular phosphatase in the proximal tubules[15,55]. This has led to the postulation that defective phosphorylation may be responsible for the faulty reabsorption. Recently Clay and his coworkers[14] have isolated solitary nephrons from 2 cases of the Fanconi syndrome, one a child and the other an adult. They consistently found that the proximal convoluted tubules were abnormal in that they were short and were joined to the glomerulus by a thin, "swan-like" neck.

It seems likely that the Fanconi syndrome is but a variant of a fairly large group of disorders which have as their common denominator a defect in tubular reabsorption. At one extreme are single deficits in the handling of water, glucose, amino acids, phosphate, probably bicarbonate and possibly potassium. At the other end of the scale are cases with the complete gamut of resorptive deficiencies such as those reported by Milne and his associates[47] and Sirota and Hamerman[52]. Between the two extremes are a number of combinations of defects, one of which is the Fanconi syndrome.

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The disorders are probably best grouped into unifactoral and multifactoral deficiencies[34] (Table 1). The syndromes manifesting single defects are:

1. NEPHROGENIC DIABETES INSIPIDUS. This is extremely rare and is due to a primary defect of water reabsorption by the kidney, resulting in polyuria with urine of low specific gravity insensitive to posterior pituitary extract. Williams and Henry[60] found this defect in 7 members of one family. Their studies of renal function in one of these patients revealed that the disorder had its origin in the renal tubules.

2. RENAL GLYCOSURIA. This is the best known and most common disorder in the group. Friedman and his coworkers[31] clearly established that faulty reabsorption of glucose is responsible for this condition.

3. AMINOACIDURIA WITH NORMAL BLOOD LEVELS OF AMINO ACIDS. This occurs as a result of defective tubular reabsorption and must be differentiated from the "secondary" aminoaciduria which may result from hepatic insufficiency, poisoning with phosphorus, chloroform and uranium, increased protein catabolism occurring in wasting diseases, burns, crush injuries and hyperthyroidism and advanced renal failure[9,24]. Primary renal aminoaciduria occurs in hepatolenticular degeneration (Wilson's disease) and cystinuria.

In 1948, Uzman and Denny-Brown[59] found evidence of excessive aminoaciduria in hepatolenticular degeneration prior to the development of hepatic damage. There is an increase in the amino acids normally found in the urine[16], a pattern similar to that seen in the Fanconi syndrome[19,20,55]. Subsequent studies have demonstrated that the origin of the aminoaciduria is faulty reabsorption of amino acids by the renal tubules[16,25]. The prolonged loss of essential amino acids may be the cause of the cirrhosis of hepatolenticular degeneration. About 20% of cases of the Fanconi syndrome have been associated with cirrhosis, possibly on the same basis[1,32,55]. The relationship of the aminoaciduria to the alterations in copper metabolism in Wilson's disease is not as yet clearly understood. Recently, extensive studies

TABLE 1.—TUBULAR RESORPTIVE DEFICIENCIES

A. Unifactoral
   1. Water→Nephrogenic Diabetes Insipidus
   2. Glucose→Renal Diabetes
   3. Phosphate→Vitamin D Resistant Rickets
   4. Amino acids→Hepatolenticular Degeneration (Wilson's Disease)
      →Cystinuria
   5. Bicarbonate (?)→Hyperchloremic Acidosis (Hyperchloremic Nephrocalcinosis)
   6. Others (??)
      a. Calcium→Idiopathic Hypercalcuria
      b. Potassium

B. Multifactoral
   1. Glucose + Phosphate→Glycosuric Rickets
   2. Glucose + Amino acids
   3. Glucose + Phosphate + Amino acids→ Classical Fanconi Syndrome
      a. Water
      b. Bicarbonate
      c. Potassium
   4. Cystinosis

on this subject have been reported by Uzman[58], Stein, Bearn and Moore[54] and Bearn and Kunkel[7]. A parallelism has been demonstrated between the urinary copper and amino acid excretion. Bearn and Kunkel[7] suggest that in Wilson's disease there is an increased absorption of copper and secondary accumulation of copper in the tissues. This, they postulate, may result in cirrhosis of the liver and its accumulation in the kidney may give rise to renal tubular damage.

Cystinuria is a specific resorptive defect involving 3 similar diamino amino acids: cystine, lysine and arginine. It is familial and usually benign unless cystine, the least soluble of the 3, is precipitated in the urinary tract with resultant stone formation[22,53,61].

4. VITAMIN D RESISTANT RICKETS. This is rare but well documentated and has been shown to be due to faulty reabsorption of phosphate[12,30,42]. It cannot be attributed to the faulty reabsorption of Vitamin D from the gastrointestinal tract since the disease persists even though Vitamin D is present in the blood in high concentration[6]. This is confirmed by its refractoriness to the intravenous administration of Vitamin D[2]. Although a familial incidence is not always present, it is reported frequently enough to suggest that the disease is genetically determined[40,43].

Resistant rickets differs clinically from the usual infantile type in several ways. It persists into or may first manifest itself in childhood or adult life, while typical rickets is uncommon beyond the eighteenth month. The serum phosphorus is low in resistant rickets and its failure to rise with therapy seems to be a salient feature of the disorder. This is in decided contrast to infantile rickets where a rise in the level of serum inorganic phosphorus is one of the first signs of improvement. The clinical findings in the 2 types are similar and the differentiation can be made only when lack of response to the usual therapy is noted. Massive doses of Vitamin D may be necessary to bring about improvement, as much as 1,500,000 units daily having been required[6].

5. HYPERCHLOREMIC ACIDOSIS. This is known by a variety of synonyms including hyperchloremic nephrocalcinosis, Butler-Albright syndrome and renal tubular acidosis. Latner and Burnard[38] first suggested that this complex syndrome may be explainable on the basis of a single defect, the failure of bicarbonate reabsorption by the proximal tubules. The hyperchloremia may represent a compensatory increase in tubular reabsorption of chloride to balance the excessive bicarbonate loss. This theory of pathogenesis is but one of many proposed and further investigation of the problem is necessary. Recently Schreiner, Smith and Kyle[51] have reviewed the subject and demonstrated for the first time that this disease can have a familial basis.

6. OTHER UNIFACTORAL DEFECTS. There is a possibility that other single resorptive defects exist. Idiopathic hypercalcuria has been reported but is not well documented[4].

Since hypokalemia has been reported as part of multifactoral resorptive defects, it would be attractive to place familial periodic paralysis, in which hypokalemia is the only detectable abnormality, into the group of unifactoral defects. There is, however, no evidence to support this. Talbott[56] found no appreciable rise in potassium excretion before or during attacks. Quantitative studies by Danowski[17] and his coworkers seem to show a transfer of extracellular potassium into the intracellular phase associated with the onset of muscular paralysis.

The multifactoral resorptive deficiencies are more difficult to define.

Theoretically any combination of defects might occur, but actually only a few have been noted clinically.

1. "GLYCOSURIC RICKETS", a syndrome of renal glycosuria and Vitamin D resistant rickets has been reported. In their extensive review of the Fanconi syndrome, McCune, Mason and Clarke[44] collected 24 instances of hypophosphatemic rickets with nondiabetic glycosuria. In none of these was aminoaciduria investigated and it seems probable that some, if not all of these cases actually represented the complete Fanconi syndrome. In his recent review of rickets and osteomalacia due to renal tubular defects, Dent[21] could find only 4 cases of defective phosphate and glucose reabsorption. All 4 patients were adults and one had a brother with Vitamin D resistant rickets. The term Milkman's syndrome has been used to include a variety of conditions characterized by osteomalacia and linear "pseudofractures." Some of the cases reported under this name have had renal glycosuria and probably represent tubular resorptive deficiencies of both glucose and phosphate[10,26,46].

2. AMINOACIDURIA PLUS RENAL GLYCOSURIA. Of a recent series of 6 cases of hepatolenticular degeneration studied by Cooper and his coworkers[16], 3 had renal glycosuria, and of 17 cases of Wilson's disease reported by Bearn and Kunkel[7], renal glycosuria were present in 5. Pitts[48] has demonstrated that the amino acid reabsorptive process of the renal tubules is distinct from that of glucose reabsorption. However, it is interesting to note that in cases of Fanconi syndrome studied by Dent[19,55] and Milne, Stanbury and Thomson[47] the glycosuria paralleled the levels of the aminoaciduria.

3. FANCONI SYNDROME. In addition to the classical triple resorptive deficiencies of glucose, phosphate and amino acids, patients with the Fanconi syndrome may have defects in the reabsorption of water and may manifest the characteristics of hyperchloremic acidosis and hypokalemia.

Polyuria has been associated with some cases of the Fanconi syndrome[32,34,41,44,47]. The volume of urine in these cases has been too great to be caused by an osmotic diuresis produced by glycosuria or organic aciduria. In none of them, however, was the urinary specific gravity as low as that reported in diabetes insipidus. The polyuria reported in the case described by Linder, Bull and Grayce[41] was unaffected by pituitrin injections.

Hyperchloremic acidosis has been reported in association with the Fanconi syndrome by Sirota and Hamerman[52] Milne, Stanbury and Thomson[47] and Salassa and his coworkers[49]. The investigations of these groups lend support to the work of Latner and Burnard[38] suggesting that faulty bicarbonate reabsorption may be the cause of hyperchloremic acidosis.

Hypokalemia has been an occasional accompaniment of the Fanconi syndrome[5,44,47] but only a few patients have been investigated in respect to serum potassium levels. Hypokalemia is suggested by the clinical picture presented by some patients with Fanconi syndrome. The patient of Stowers and Dent[55] experienced "collapsing incidents in which his legs seemed to give way under him." The mistaken use of insulin carries a considerable risk of induced hypokalemia as does the glucose tolerance test. Fanconi[28] recorded symptoms and electrocardiographic changes suggesting hypokalemia following glucose tolerance tests in 2 of his patients. Debre and his associates[18] reported death following a glucose tolerance test. Increased potassium excretion was found in the cases described by Sirota and Hamerman[52] and Milne and his coworkers[47]. Both were

patients with hyperchloremic acidosis and the potassium diuresis was thought to be a renal mechanism for sparing sodium. In several adult cases of hyperchloremic acidosis, continued loss of potassium has led to the typical features of hypokalemia[11,38,40]. It is of interest to note that in the patient reported by Sirota and Hamerman[52] osmotic diuresis induced by glucose resulted in an increase in potassium excretion. This suggests that faulty reabsorption of potassium may have been present.

4. CYSTINOSIS. This is considered by most observers to be a variant of the Fanconi syndrome characterized by the widespread deposition of cystine crystals in many organs. Renal glycosuria and hypophosphatemia may be present. Cystinuria is not a constant finding in cystinosis. Less than 25 cases of cystinosis have been reported, most of them in the European literature, and cystine was demonstrated in the urine of only 5 of them. Clarke and Jackson[13,35] and King and Lochridge[36] have reported cases in this country.

The Fanconi syndrome is rare in the adult, only a few such cases having been described. In their recent review Sirota and Hamerman[52] mentioned 11 previously recorded patients over the age of 13 and presented one of their own. Of these 11 cases, those reported by Milkman[46], Edeiken and Schneeberg[26], Hunter[33] and Fletcher[29], though suggestive of the Fanconi syndrome, were not studied for aminoaciduria and, therefore, cannot be considered to be fully documented. This is also true of similar adult cases not included in this review which were previously reported as "glycosuric rickets" or Milkman's syndrome[3,39,45,46]. The first well documented adult patient appears to have been that of Stowers and Dent[55] in 1947. Cases were subsequently described by Cooke and his associates[15], Linder, Bull and Grayce[41], Milne, Stanbury and Thomson[47], Anderson, Miller and Kenny[5] and Lambert and de Heinzelinde de Braucourt[37]. Since the review by Sirota and Hamerman[52], an additional adult case has been reported recently by Dragsted and Hjorth[23].

The following patient, the oldest fulfilling the diagnostic criteria of the Fanconi syndrome, is presented at this time.

Case Report. C. L., a 56-year-old white male, was admitted to the Philadelphia Veterans Administration Hospital on February 26, 1953. He had been well until 2 months prior to admission, at which time he developed progressively incapacitating pain in the low back associated with a 15-pound weight loss. Review of symptoms by systems, past medical history and family history were unrevealing. On physical examination, there was tenderness to percussion over the lumbar spine and limitation of back motion. A hard, walnut-sized lymph node was felt in the left posterior cervical triangle. The remainder of the physical examination was not remarkable.

Laboratory data are outlined in Table 2. Routine blood counts were normal and the serological test for syphilis was negative. Many urinalyses were normal except for varying degrees of glycosuria. The urinary sugar was identified as glucose and 24-hour excretion averaged 10 gm. This occurred in the presence of fasting blood sugar levels of 77 to 107 mg. per 100 cc. The glucose tolerance test was normal on 2 occasions. Renal function as measured by the usual clinical methods was normal. There was no polyuria. The blood calcium level was normal despite which, even on an intake of 40 to 50 mg. of calcium daily, there was a 4+ Sulkowitz test and an average of 140 mg. of calcium were excreted daily. The serum inorganic phosphorus was consistently low. Sodium, potassium, chlorides, CO_2-combining power and acid phosphatase were normal. The alkaline phosphatase was slightly elevated. The blood amino acid level was normal despite which there was a several fold increase in amino acid excretion above normal. Unfortunately, the specific amino acids excreted were not identified by paper chromatography or biochemical means.

Roentgenograms revealed generalized bony demineralization (Fig. 1). Several linear fractures were noted in the ribs and scapulae.

Compression of several lumbar vertebrae was present (Fig. 2). Electrocardiograms were within normal limits.

A cervical lymph node biopsy performed shortly after admission revealed an undifferentiated adenocarcinoma. All attempts to locate the primary site of the lesion were unsuccessful. The patient's course was progressively downhill and he expired 10 weeks after admission. Neither uremia nor acidosis developed.

Examination at necropsy revealed carcinoma of the body of the pancreas with metastases to lymph nodes, liver, lungs and heart. The kidneys were grossly normal. The parathyroids were not hypertrophied.

Microscopic examination confirmed the diagnosis of adenocarcinoma of the pancreas.

TABLE 2.—LABORATORY DATA ON REPORTED CASE

Hemoglobin	12 to 14 gm./100 cc.
White blood count	4700 to 9600/c.mm.
Urine	
Specific gravity	1.013 to 1.025
pH	5.0
Proteinuria	0
Glycosuria	0 to 4+
Sediment	Negative
24 hour output	700 to 2000 cc.
Sulkowitz test	4+
PSP excretion	
15 minutes	25%
30 minutes	55%
60 minutes	10%
Total 1 hour	90%
Urea clearance	98% of normal
Plasma or serum concentrations	
Nonprotein nitrogen	25 mg./100 cc.
Urea nitrogen	10 to 13 mg./100 cc.
Creatinine	1.8 mg./100 cc.
Uric acid	2.8 mg./100 cc.
Amino acids	4.0 mg./100 cc.
Fasting glucose	69 to 107 mg./100 cc.
Albumin/globulin	4.85/1.30 gm./100 cc.
Sodium	140 mEq./L.
Potassium	4.5 mEq./L.
CO_2 combining power	23 mEq./L.
Calcium	10.9 to 11.4 mg./100 cc.
Inorganic phosphorus	2.1 to 2.8 mg./100 cc.
Acid phosphatase	2.4 Bodansky units
Alkaline phosphatase	6.4 Bodansky units
Cephalin flocculation	Negative
Thymol turbidity	0.3 units
Thymol flocculation	Negative
Bromsulfalein retention	3.5%
Glucose tolerance test	
Fasting	77 mg./100 cc.
½ hour	146 mg./100 cc.
1 hour	160 mg./100 cc.
2 hours	110 mg./100 cc.
3 hours	86 mg./100 cc.
24 hour glucose excretion	10 gm.
24 hour calcium excretion	140 mg.
24 hour amino acid excretion	
Free amino acids	37.4 millimol.
Alpha amino nitrogen	523 mg.

Fig. 1.—Roentgenogram of femur showing marked demineralization.

Fig. 2.—Roentgenogram of lumbar spine showing marked demineralization and vertebral compression.

Degenerative changes were present in the proximal tubules of the kidney (Fig. 3), but the glomeruli appeared relatively normal. Sections of bone revealed generalized osteomalacia. No bony metastases were present. The liver was not remarkable except for the presence of metastases. There was no apparent relationship between the underlying disease and the degenerative changes in the kidney and the manifestations of the Fanconi syndrome.

Therapy and Prognosis. Therapy of the Fanconi syndrome should be directed toward the correction of the deficiencies produced by the resorptive defects. Recently Salassa and his coworkers[49] found that large doses of Vitamin D (400,000 units daily) favorably modified the hypophosphatemia and high renal clearance of phosphate, the glycosuria, the aminoaciduria and the hyperchloremic acidosis in 2 young patients with the Fanconi syndrome. They suggested that this is in accord with Zetterström's[62] observations on the relationship between Vitamin D and the activation of alkaline phosphatase in the renal tubules.

Alkalinizing regimens have been reported to produce favorable results in the treatment of hyperchloremic acidosis. Sodium citrate, citric acid and sodium bicarbonate are usually recommended for this purpose.

If hypercalcuria is present, calcium should be administered orally. Potassium is indicated if hypokalemia exists.

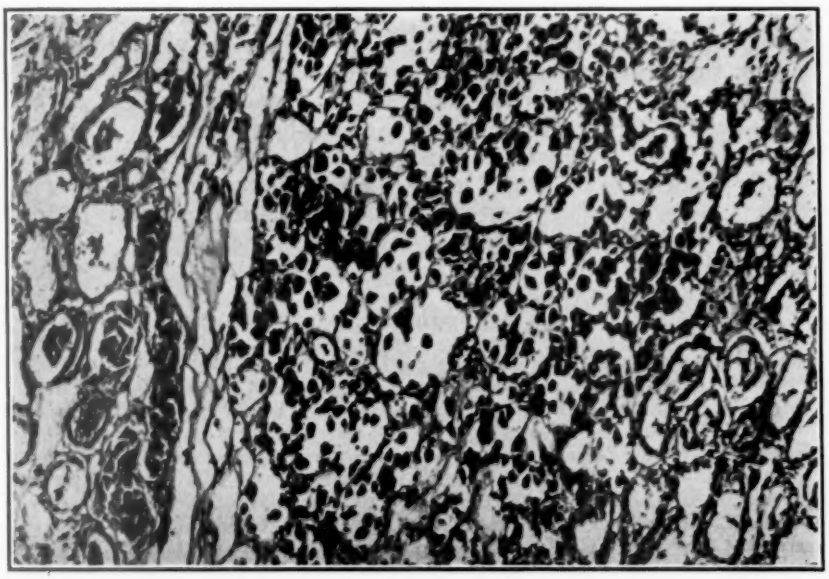

Fig. 3.—Photomicrograph of kidney showing degenerative changes in the proximal tubules.

Anderson and his coworkers[5] found that the administration of testosterone produced a decrease in the aminoaciduria. This has some basis in animal experiments in which testosterone has produced enlargement of the kidney and hypertrophy of the proximal convoluted tubules in certain species.

The prognosis of the Fanconi syndrome is variable. The juvenile cases, prior to 1943, reviewed by McCune, Mason and Clarke[44] had a high death rate. Death occurred as a result of de-

hydration, acidosis or intercurrent infection. The deposition of cystine in vital organs is a serious complication and appears invariably to be fatal.

With a better understanding of the mechanism of the Fanconi syndrome the prognosis appears to have improved. Only a few patients have been followed for an appreciable length of time. The patient of Sirota and Hamerman[52] has been followed for 5 years and those of Salassa and his coworkers[49] for 18 months. The latter have improved remarkably on Vitamin D.

Summary. The Fanconi syndrome results from a defect in function of the proximal convoluted tubules of the kidney leading to faulty reabsorption of glucose, phosphate, amino acids and occasionally water, bicarbonate and potassium.

It appears likely that the Fanconi syndrome is one of a fairly large group of disorders which have as their common denominator faulty resorptive function of the proximal tubules. Unifactoral deficiencies involving faulty reabsorption of water, glucose, amino acids, phosphate and possibly bicarbonate result in the clinical syndromes of nephrogenic diabetes insipidus, renal glycosuria, Wilson's disease (hepatolenticular degeneration), cystinuria, and possibly hyperchloremic acidosis. Multifactoral deficiencies are more difficult to define but appear to include the Fanconi syndrome and cystinosis.

The Fanconi syndrome is rare in the adult. A man, 56 years of age, is presented who fulfilled the diagnostic criteria of the Fanconi syndrome.

Some comments have been made on the therapy and prognosis of the Fanconi syndrome.

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MYELOID METAPLASIA AS A SEQUELA OF POLYCYTHEMIA

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The discussion of myeloid metaplasia has to a singular degree the disadvantages of confusion in terminology and varying conceptions of its nature. Block and Jacobson[1] have estimated that the subject has appeared in the literature under 25 different names. Its nosologic position is also under dispute, particularly its relationship to leukemia. A number of contributions have approached the topic from the viewpoint of the osteosclerotic lesions which have been noted as concomitant findings with leukoerythroblastic anemia. Among the more comprehensive articles in this category is that of Rosenthal and Erf[12]. They record that Heuck[7] first described myelofibrosis in 1879; the patient in this report had splenomegaly, leukocythemia, anemia and extramedullary hemopoiesis. In 1940, Jackson, Parker and Lemon[9] described a series of cases which they grouped under the heading of agnogenic myeloid metaplasia. They mentioned an occasional early polycythemia in this syndrome and described the bone marrow as normal, aplastic, hyperplastic or fibrotic. They considered that the picture was definitely not leukemic.

Because of the marked alterations of the peripheral leukocytes, particularly in the granular series, two conflicting opinions on the nature of myeloid metaplasia have arisen. Heller, Lewisohn and Palin[6] take a strong stand as to the leukemic nature of the disturbance. With equal conviction many students of the subject have maintained that these changes are leukemoid in type, particularly in view of the normal maturation of myelogenous cells in the bone marrow. The former contend that the marrow may at times not clearly reflect the leukemic process. All authors are unanimous in agreement on the serious outcome of splenectomy or even splenic irradiation.

While observers have been preoccupied either in the separation of this syndrome from, or its identification with, leukemia, sufficient attention has not been given to a comparable pathologic state which accompanies or forms a sequela of polycythemia. The resultant syndrome is morphogenetically comparable if not etiologically related to some instances of myeloid metaplasia. This nexus clearly exists in many published reports, though obscured by the use of alternative designations for the finally evolved disease. Senator[13], in 1911, and Hirschfeld[8], in 1912, described "myeloid transformation" in the spleens of some patients with polycythemia. Lang[10] considered these cases as instances of extramedullary hematopoiesis involving structures which generated myeloid tissue in the embryo. One of the first clear-cut studies to link up polycythemia with

myeloid metaplasia was that of Minot and Buckman[11]. They found that extramedullary hematopoiesis may occur in long-standing polycythemia and called attention to the leukemoid blood picture and the appearance of megakaryocytic fragments in the peripheral blood. In 3 patients the considerable increment in spleen size occurred in the late anemic phases of the disease. In one necropsied patient the spleen presented striking myeloid transformation which was difficult to distinguish from myeloid leukemia. Their final opinion was that the process did not resemble leukemia but possibly bore a relationship to the latter.

Tinney, Hall and Giffin[15] found a leukemoid reaction in 15 patients with polycythemia in whom the spleen was greatly enlarged. In 5 of these the changes were so marked that the blood smears simulated myelogenous leukemia. Four of these 5 patients had survived periods varying from 12 to 23 years after the diagnosis of polycythemia had been made. The histology of the spleen is not mentioned in these articles.

In their paper on chronic nonleukemic myelosis, Carpenter and Flory[2] describe a rare initial polycythemia. They consider the process distinct from leukemic myelosis due to the diversity of cell types in the metaplastic organ and bone marrow and the almost constant presence of nucleated red cells in the peripheral blood. The frequency of osteosclerosis and myelosclerosis and the absence of characteristic leukemic infiltration are also emphasized. They quote the views of Vaughan and Harrison[16]. The latter proposed a concept that a single common stimulus might provoke a multipotential reaction in the reticulum, affecting osteoblasts, fibroblasts and hemopoietic cells.

Dameshek[3] has described the late stages of polycythemia associated with myelofibrosis, marked splenomegaly and leukoerythroblastic anemia. He states that this picture resembles Jackson, Parker and Lemon's "agnogenic myeloid metaplasia" and similar syndromes described by other authors under various names. Dameshek[4] has also proposed a comprehensive hypothesis which groups and tabulates differences and transitions among a variety of myeloproliferative disorders; he postulates that the common link is some stimulus, at present unknown.

In their study of nonleukemic myelosis, Heller *et al.*[6] have occasion to consider the mechanism of extramedullary hemopoiesis in their cases though polycythemia was not a factor. They criticize the supposition that deficient hematopoietic activity in the marrow produces the extramedullary myeloblasts as a compensatory process. They state that in most cases the cellular reaction in the spleen was mainly leukopoietic. In this regard it is noteworthy that Hirschfeld[8] commented on the predominantly leukoblastic aspect of the myeloid transformation in polycythemia. In reconciling these views we prefer to restrict the compensatory types to those instances which are related to bone marrow injury by known chemical exposure.

The following case histories illustrate the development of myeloid metaplasia of the spleen in relationship to polycythemia. In the first patient the syndrome supervened upon a long initial phase of polycythemia. The second patient did not come under clinical study until late in the course of the disease and the blood pattern was no doubt accentuated by splenectomy.

Case Reports. CASE 1. R. K., a Jewish female, aged 54 years, was admitted to the Graduate Hospital on November 27, 1947. She complained of loss of weight, weakness and shortness of breath. On examination, pallor, gallop rhythm and crepitant râles throughout both lung fields were noted; the spleen

extended down to the brim of the pelvis and the liver to the level of the umbilicus. A chronologic summary of her past history is as follows:

She was first studied under hospital conditions in March, 1953, at the age of 42. Her complaints at this time were redness of the skin, headache and vertigo. On examination, she had a blood pressure of 135 mm. Hg systolic and 95 mm. Hg diastolic, the spleen was palpable and tender 3 cm. below the left costal border. Blood examination revealed the hemoglobin to be 140%, erythrocytes 85%, the erythrocytes numbered 4,250,000, and the white blood cells 16,700 per c. mm. The differential count showed 80% segmented neutrophils with 2% premyelocytes. The bone marrow revealed normal maturation of the myelogenous series with a shift to the right and moderate erythropoietic activity.

She was re-admitted in November, 1945, with a history of loss of 10 pounds in weight during the 2 weeks prior to admission. The spleen was enlarged to the level of the umbilicus; the liver was not definitely enlarged. The hemoglobin was 9.5 gm. per 100 cc.,

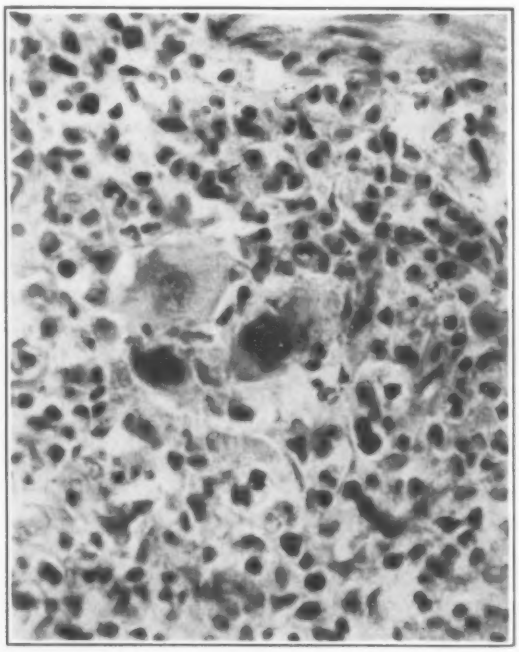

Fig. 1.—Biopsy of spleen of R. K. Large cells in center are megakaryocytes.

numbered 7,500,000 per c.mm. and leukocytes 12,800 per c.mm.; differential count of the white cells was normal. She was treated with phenylhydrazine and received Roentgen-ray therapy over the spleen, iliac bones and femora. About 3 weeks later the hemoglobin had fallen to 92%, and the erythrocytes to 4,850,000 per c.mm., though the leukocytes were 16,000 per c.mm.

She was re-admitted in 1943, at the age of 50 with the complaint of fatigability without loss of weight. The spleen was now 6 cm. below the left costal border and the liver slightly palpable. The hemoglobin was erythrocytes 3,700,000, and leukocytes 25,000 per c. mm. Mean corpuscular volume of the red cells was 90 cubic microns and mean corpuscular hemoglobin concentration was 26.5%. No osteosclerotic lesions were found on roentgenograms of the skull and pelvis. A biopsy of the spleen was considered, but the occurrence of undue bleeding following extractions of teeth discouraged this plan.

On December 23, 1946, a laparotomy with biopsy of the spleen was done by Dr. S. Levine. Dr. Helen Ingleby gave the following pathologic report:

The normal architecture of the spleen is

almost lost. The occasional remains of a Malpighian body and walls of some of the sinuses can be made out. Some areas show fibrosis. Elsewhere the pulp presents numerous abnormal cells among which are many very large giant cells. The giant cells tend to have centrally placed nuclei. Many eosinophil cells are found in some areas. Large and small mononuclear cells and reticulum cells are also found.

With Wright's stain the giant cells are found to be definitely megakaryocytes. They possess very large irregular sometimes indented nuclei, dotted with dark chromatin granules and showing a chromatin network. There is a large amount of pale cytoplasm showing very fine bluish granules on a pinkish background. In addition one finds all stages of development from myeloblasts to adult cells of the granular series. There are many characteristic eosinophilic myelocytes as well as adult eosinophils. All stages of development from erythroblasts to normal red cells are seen. Many macrophages are present, some containing green material, probably pigment derived from blood. Much of this pigment is also seen extracellularly.

Convalescence following this biopsy was uneventful except for the recurring severe anemia which was controlled by frequent transfusions. At the time she came under our care at Graduate Hospital she had had 37 transfusions. The objective physical findings on this admission, November 27, 1947, are noted above.

Hematologic studies revealed the hemoglobin to be 4 gm. per 100 cc., erythrocytes 1,720,000 per c. mm., leukocytes 41,700 per c. mm., hematocrit 12% packed red cells. Peripheral blood smears revealed a hypochromic microcytic anemia with evidence of active regeneration. There was marked anisocytosis with a tendency to microcytosis, poikilocytosis and polychromatophilia with a generalized hypochromia. An occasional normoblast was seen. A neutrophilic leukocytosis was noted, there were marked toxic changes of the neutrophils, but very little evidence of immaturity. Platelets were present, but reduced in number. Bone marrow was aspirated from the sternum. Direct smears revealed cellular preparations with a predominance of mature neutrophils. Less than the usual number of immature granulocytes were seen and very few nucleated erythrocytes were encountered. Those seen were all in the normoblastic line. A few normal megakaryocytes were found. Subsequent blood counts, examination of blood and bone marrow smears revealed no new information. The erythrocyte count and hemoglobin increased slowly following transfusions. The leukocyte counts ranged between 12,800 and 65,000 per c. mm. with a marked preponderance of toxic neutrophils, only an occasional myelocyte being seen. The erythrocyte picture remained the same. Reticulocytes ranged between 3.5 and 10.6%. The blood proteins were 6.4 gm. per 100 cc. total with 3.79 gm. per 100 cc. albumin and 2.61 gm. per 100 cc. globulin. The blood uric acid was 8.1 mg. per 100 cc. Roentgenograms of the skull and pelvis revealed no abnormality.

Although almost moribund on admission, the patient responded to repeated transfusions and other symptomatic measures. She was discharged February 16, 1948, her last blood count being: hemoglobin 7.5 gm. per 100 cc.; erythrocytes 2,810,000; leukocytes 18,000. She was then followed as an outpatient and transfused with concentrated red cells at intervals of 2 or 3 weeks.

Because of dyspnea and marked weakness the patient was readmitted May 10, 1948. Her physical findings were essentially unchanged. There was marked pallor, the spleen occupied the entire left upper and lower quadrants and the liver extended slightly below the level of the umbilicus. On this admission, studies revealed hemoglobin 4.5 gm. per 100 cc., erythrocytes 1,420,000 per c. mm., and leukocytes 43,500 per c. mm. The peripheral blood smears were essentially unchanged. Aspirated bone marrow was hypocellular, but otherwise similar to that noted previously. Total proteins were 5.60 and 5.20 gm. per 100 cc. with albumin 3.52 gm. and globulin 2.08 gm. on the first occasion and 2.59 gm. and 2.61 gm. per 100 cc. on the second. Blood uric acid was 7.7 mg. per 100 cc., blood urea nitrogen 10 mg. per 100 cc. and the serum van den Bergh revealed negative direct and 0.3 mg. of bilirubin per 100 cc. on indirect reaction.

Treatment again was symptomatic with transfusions of concentrated red cells. On June 8, 1948, she was discharged with hemoglobin 11 gm. per 100 cc., erythrocytes 3,840,000 and leukocytes 14,600 per c. mm. She was advised to return to the clinic for further transfusions. This she did not do and died at home on September 8, 1948.

Necropsy Report. Gross examination by Dr. Joseph E. Imbriglia (relevant findings only are given): An enormously enlarged spleen weighing 2350 gm. was present. Splenic parenchyma was firm and hard; normal architecture was destroyed. Areas of fibrosis were noted throughout the parenchyma. Lymph nodes along the hilar region of the

spleen were enlarged 6 to 10 times their normal size and were firm in consistency. The surface had a bluish gray color; on section normal lymph node architecture was lost. The parenchyma was gray in color interspersed with areas of reddish yellow. The latter were pinhead in size and were scattered uniformly throughout the lymph node. The para-aortic lymph nodes showed similar changes. The liver weighed 3680 gm. Interspersed in the liver parenchyma were noted pinpoint reddish yellow areas which stood out prominently against the pale hepatic parenchyma. The bone marrow of the sternum, vertebrae and ribs was examined. It was of a pink color and not grossly sclerotic.

Microscopic description by Dr. A. Valdés-Dapena:

Lymph Nodes: The normal monotonous predominance of lymphocytes is replaced by an extremely varied assortment of cells. Among these the most striking are large multinucleated elements which correspond to megakaryocytes. These are not strictly normal in all cases, overly large vesicular nuclei being in evidence in many of the cells. Other cells included eosinophilic and neutrophilic granulocytes in all stages of maturation.

Spleen: The spleen is recognized as such in these sections by the presence of characteristic connective tissue trabeculae. For the rest, the normal landmarks are erased. There is very marked engorgement of the sinusoids. Among the masses of crowded red cells, nests of developing nucleated cells of all types stand out. These are frequently located within sinusoids. Megakaryocytes and myeloid cells are very prominent. All degrees of maturation are observed. Mature cells are abundant.

Sections of Bone Marrow: The examination of the sections of bone marrow taken at necropsy show a diffuse hyperplasia. The cells are not packed as they would be in a leukemia but fatty tissue has been completely crowded out of the picture. On close examination there appears to be a predominance of fairly large cells of the type which usually represent young elements but there is a fairly well distributed participation of red and white cells. Megakaryocytes are likewise numerous. This could be described as a massive hyperplasia of all elements of the bone marrow. There is no evidence of osteosclerosis in this section. This of course does not rule out the possibility of areas of sclerosis elsewhere in the osseous system.

General Summary: Myeloid metaplasia of spleen, liver, left kidney and lymph nodes of the splenic hilar and para-aortic regions.

CASE 2. M. T., a Jewish male, aged 49 years when first seen by us in April, 1944, complained of spells of vertigo and occasional attacks of unconsciousness. He had been well until October, 1943, when he noticed a feeling of pressure under the ribs on the left side. An enlarged spleen was noted in January, 1944, and was removed in February of the same year. The histologic sections of the spleen and the blood counts were kindly supplied by Dr. David Meranze of Mt. Sinai Hospital who made the pathologic diagnosis of myeloid metaplasia of the spleen. The preoperative blood count revealed the hemoglobin to be 15.95 gm. per 100 cc., leukocytes numbered 14,900 to 15,300 per c. mm. and platelets 260,000 per c. mm. Red cell counts were not available.

The histologic sections of the spleen revealed altered architecture so that the Malpighian bodies were not noticeable, some cords of Billroth remained. The usual lymphoid pulp was replaced by cells of the myeloid series particularly metamyelocytes, polymorphonuclear neutrophils and eosinophils and a few myelocytes. Erythropoiesis was slight and some hyperemia and reticular hyperplasia were present. Slight fibrosis and pigment granules were present. Megakaryocytes were seen.

On examination in April, 1944, he presented the typical facies of polycythemia, a normal blood pressure and hepatic enlargement of two finger breadths under the costal border. His blood counts during the next 3 years fluctuated between moderate polycythemic normal and anemic levels. The highest red cell count in this period was 7.0 million, and the lowest 3,020,000 per c. mm. The lowering of the red cell count could be attributed in the main to the use of irradiation therapy, though in one acute episode the fall was associated with tarry stools and signs suggesting internal hemorrhage. During his entire observation the peripheral blood was leukemoid with white cells ranging from 39,000 to 84,000 per c. mm.; typical differential counts were neutrophils 85%, metamyelocytes 7%, premyelocytes 2%, myelocytes 3% and lymphocytes 3%. Normoblasts were usually present in moderate number; platelets numbered 656,000 per c. mm.

Bone Marrow Examination. Fairly cellular; neutrophils 42%, eosinophilic polynuclears 3%, neutrophilic metamyelocytes 28%, eosinophilic metamyelocytes 1%, myelocytes 2%, lymphocytes 3%, monocytes 2%, normoblasts 11%, erythroblasts 6% and megakaryocytes 2%. The appearance was not that of leukemia

particularly in view of the shift of the myeloid series to the right.

Course. When not controlled by irradiation the patient would lapse into the polycythemic phase ushered in by increased ruddiness of the face, bleeding from the gums and the cerebral manifestations described above. He received transfusions in the anemic phases. He died suddenly at home and necropsy was not obtained.

Comment: The case histories of the two patients here presented are not dissimilar to those described by the authors quoted above. The transition from overt polycythemia to the spent and anemic stage or the late leukemoid phase associated with myeloid change in the spleen is fairly clear if followed consecutively over a period of years in the same patient as in Case 1. In contrast the difficulty of constructing such a course in retrospect when the patient is first seen in the late stage is brought out in Case 2. The distortion of the clinical picture induced by splenectomy in the latter cannot be denied.

Discussion. A suitable classification is important in the clarification of any subject. For reasons stated at the outset, this has been particularly difficult in myeloid metaplasia. The classification proposed by Block and Jacobson[1] is among the more recent and satisfactory ones. They divide the condition into two general types, primary and secondary. In the secondary group are placed cases with metastatic carcinoma, marble bone disease, tuberculosis, myelofibrosis and osteosclerosis. The combination of osteosclerosis and polycythemia is included in their table of 12 cases of myeloid metaplasia. They, therefore, place this syndrome in the secondary group.

Since the myeloid metaplasia of the patient, R. K., of our report and many others in the literature stems as a sequela rather than a complication of polycythemia we would prefer to make a special category of this type. The classification which would be a slight modification of that of Block and Jacobson, would be as follows:

1. Primary
2. Secondary
   a) probably related to chemical exposure
   b) metastatic carcinoma with invasion of bone marrow
   c) tuberculosis
   d) marble bone disease
   e) osteosclerosis unrelated to polycythemia
3. As a sequela of polycythemia (with or without osteosclerosis)

The primary group is self-explanatory and would make the use of the term "agnogenic", unnecessary. This is particularly desirable since some of the earlier reported instances of agnogenic myeloid metaplasia were later shown to be related to exposure to industrial solvents such as benzol. The latter can therefore be conveniently placed in the secondary group.

The osteosclerotic subdivision of the secondary group offers a special difficulty due to the overlapping of such topics as osteosclerotic anemia and myelofibrosis either of which has been described with late stages of polycythemia and also in patients with no history of polycythemia. In addition, myeloid metaplasia may or may not have been associated with these bone lesions. Some writers have discussed this as a special topic. In the main these articles deal with myelofibrosis and osteosclerosis from the viewpoint of primary bone disease. Where polycythemia has also been noted it has been included as a subgroup of the bone disease. In those instances in which osteosclerotic lesions have supervened in the late stages of polycythemia a compensatory or vicarious type of extramedullary hemopoiesis has not seemed surprising. It has been accept-

ed as an expression of the unrestrained stimulus to all components of the marrow with a reversal to an embryonal form of blood formation. It is possible that the same explanation holds for such cases where a hypoplasia of the marrow occurs even in the absence of myelosclerosis or myelofibrosis. However, in our patient (R. K.) though the marrow at necropsy had the gross appearance of hypoplasia, marrow aspirations in the last few months of her illness showed satisfactory erythropoiesis and granulopoiesis.

In other instances, the vigor of the marrow seemed unimpaired and appeared red and hyperplastic. This occurred in a patient, a woman aged 42 years, described by Stone and Woodman[14]. She was studied over a period of 13 years and exhibited 3 phases: 1) original polycythemia; 2) transitory secondary anemia with leukocytosis; 3) terminal leukoerythroblastic anemia. She exhibited no bone lesions in life by Roentgen-ray examination. At necropsy, red marrow was present along the entire length of the femur and the ribs also showed active red marrow. The spleen weighed 870 gm. and exhibited foci of erythropoietic and granulopoietic tissue with scanty megakaryocytes.

The present phase of interest in hypersplenism has been reflected in a more positive approach to the management of the late stages of myeloid metaplasia. In such instances, manifestations of hypersplenism such as hemolytic anemia or thrombocytopenia may endanger the patient's life. The former discouraging opinions as to the advisability of splenectomy mentioned at the outset may have to be modified. Recently, Green, Conley, Ashburn and Peters[5] have reviewed the literature and their own records. On the basis of this analysis they consider that splenectomy may be useful in some cases where thrombocytopenia has appeared in the late stage of myeloid metaplasia. The operation is less effective in ameliorating hemolytic anemia. We also believe that this decision may have to be made in such complications as marked thrombocytopenia, though confined to those patients in whom the bone marrow is active and contains adequate numbers of immature megakaryocytes. In critically ill patients with hemolytic syndromes, the use of Roentgen therapy over the spleen as an emergency measure may be considered. We have occasionally employed this method in critical hemolytic syndromes of the lymphomas and leukemias as a temporary expedient until conditions warrant a more definitive procedure.

In some patients in whom the myeloid metaplasia has seemed to be in response to marrow encroachment from osteosclerosis, there has appeared on closer analysis a disparity between the extensive extramedullary hemopoiesis and rather moderate bone changes. We have reason to believe, though it is not susceptible to proof, that this may characterize some cases of polycythemia. However, Block and Jacobson[1] hold a contrary view. They believe that the histologic and clinical manifestations of myeloid metaplasia are essentially similar despite varied etiologic factors.

Finally as a practical diagnostic consideration we believe that myeloid metaplasia as a sequela of polycythemia is suggested by the following circumstances:

a) A prolonged history of polycythemia, in some instances 10 to 20 years.

b) Massive splenomegaly.

c) A leukemoid blood picture with normoblasts and bizarre or giant platelets in the peripheral blood smears; anemia is usually present except in those instances where splenic puncture

has revealed the syndrome during the polycythemic stage.

d) Osteosclerosis.

e) The bone marrow exhibits a shift to the mature side of the granular series with no leukemic hiatus or shift to the immature components as in leukemia.

Summary. The subject of myeloid metaplasia is discussed with particular reference to the type which appears as a late sequela of polycythemia.

The case histories of two patients are described. One of these, a woman, presented the manifestations of polycythemia vera at the age of 42 and was studied at intervals for 13 years thereafter. Myeloid metaplasia and massive splenomegaly were found to be present in the last 2 years of her life. Widespread myeloid metaplasia was found at necropsy.

A working classification of myeloid metaplasia is proposed based upon that of Block and Jacobson with slight modifications. It makes the use of the term "agnogenic" unnecessary; it integrates myeloid metaplasia with the subject of myelofibrosis and osteosclerosis.

In a patient with long-standing polycythemia, the possibility of myeloid metaplasia is suggested by: massive splenomegaly; a leukemoid blood picture with anemia, normoblasts and bizarre or giant platelets in the peripheral blood; osteosclerosis. Leukemia is ruled out by bone marrow examination which shows a shift to the mature side of the granulocyte series and no leukemic hiatus. Splenic biopsy or puncture may confirm the diagnosis.

The former teaching that splenectomy or irradiation to the spleen is harmful may in an occasional instance be altered by the complication of hypersplenism.

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STUDIES ON THE CONTROL OF HYPERTENSION BY HYPHEX. III. PHARMACOLOGICAL AND CHEMICAL OBSERVATIONS ON 1-HYDRAZINOPHTHALAZINE

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It has been shown that continuous oral administration of a combination of hexamethonium chloride and 1-hydrazinophthalazine† lowers the blood pressure of hypertensive patients for considerable periods of time[10,11,14,15]. Moreover, hydrazinophthalazine, a highly reactive compound, apparently causes collagen diseases[1,4,8]. Because of the possibility that direct chemical reactions with certain substances might occur in the human body the pharmacology and chemistry of this substituted hydrazine were explored.

Pharmacology. The effect of 1-hydrazinophthalazine on the potency of several pressor substances was assayed by intravenous injection into 90 anesthetized rats, a Sanborn electromanometer being used to measure blood pressure directly. The pressor activity of each was reassessed after 1 to 20 mg. of intravenous hydrazinophthalazine (Table 1). Pressor responses were similar in 8 renal hypertensive and 10 normotensive animals, although the fall of diastolic pressure induced by hydrazinophthalazine was considerably greater in the former (average 56 mm. Hg) than in the latter (average 22 mm.).

INDOLIC AMINES. The vasoactivity of *tryptamine* and *5-hydroxytryptamine* (as serotonin creatinine sulphate) was enhanced or little altered after hydrazinophthalazine had been injected. Even very large doses of the drug (60 to 80 mg. per kg.) did not completely inhibit the responses. Mere mixing of tryptamine and the agent had no effect on the vasoactivity of the former, although incubating them together at 70° C. and pH 3 reduced it. Before the hydrazine was given the pressor re-

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†Hydralazine, trade name "Apresoline" (Ciba). Hyphex refers to the combination of these two drugs used according to the method described[10,11,14,15].

TABLE 1.—EFFECT OF 1-HYDRAZINOPHTHALAZINE ON PRESSOR ACTIVITY OF VARIOUS COMPOUNDS IN RATS

Average Rise in Diastolic Pressure, mm. Hg

	Dose of Pressor Material	*After Intravenous Hydrazinophthalazine*						*Increased Amt. of Pressor Material after 20 mg. of Intravenous Hydrazinophthalazine*		*No. of Experiments*	*No. Consistent*	*Demonstrable Chemical Combination*
		0 mg.	*1 mg.*	*5 mg.*	*10 mg.*	*15 mg.*	*20 mg.*	*2 × Dose*	*4 × Dose*			
Phenylethylamine	25γ	46		17	5	1	1			2	2	No
Phenylethylamine	32γ	49		50	20	12	6	1		2	2	
Phenylethylamine	50γ	71		16						2	2	
Arterenone	7.5γ	40		26	5	8	0	0	15	4	4	No
Arterenone*	2.0γ	43		15	8	11	5			4	4	
Arterenone*	10.0γ	61		61	37	26	17			3	3	
Arterenone†	10.0γ	54		32	10	10	8			5	5	
Nor-Epinephrine	0.3γ	45		40	18	9	8	17		10	10	No
Tyramine	25γ	42		40	6					2	2	No
Tyramine	37.5γ	31		28	18	10	2	25		3	3	
Isoamylamine	400γ	28		18	17	7	3	7	30	4	4	No
Tryptamine	100γ	47		48	59	36	48	60		5	4	No
Tryptamine‡	50γ	49		51	27	21	9			6	5	
Serotonin	5γ	28		44						5	4	No
Serotonin	6γ	27		56	42	21				4	4	
Angiotonin	2 units	55		35						5	4	No
Angiotonin**	2 units	36		21						5	4	
Angiotonin‡	2 units	63		85	42	38	31			4	4	
Pherentasin	2 units	32	0							3	3	
Pervanadyl ion	200γ	27		4						8	8	Reduction to V^{+++}
Cadmium Sulfate	300γ	41		6						3	3	

* Mixed with the hydrazinophthalazine and allowed to stand 15 min. at 20° C.
† Mixed with the hydrazinophthalazine, made to pH3 for 15 min., and neutralized, all at 20° C.
‡ Mixed with the hydrazinophthalazine, made to pH3, heated to 70° C. for 5 min., cooled, and neutralized.
** Made to pH3, heated to 70° C. for 5 min., cooled, and neutralized.

sponse to serotonin was irregular in 6 normotensive rats, an initial depression being followed by a return to control levels or a rise above them (average diastolic rise 10 mm. Hg); however in 6 hypertensive animals the depression occurred after only 1 of 11 injections and the pressor response was uniform (average diastolic rise 46 mm.). Tryptamine was more regularly pressor.

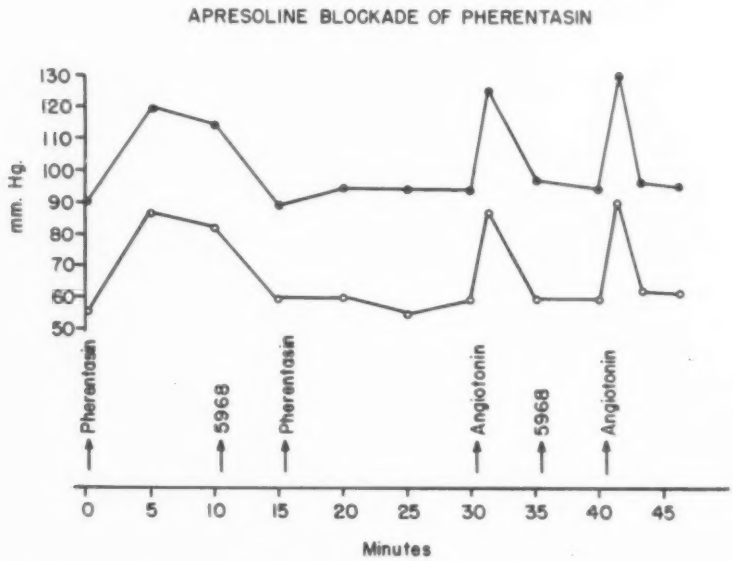

Fig. 1.—At 0 minutes, after a control period of 20 minutes, 1.0 unit of pherentasin previously found active was injected intravenously into a rat, with the response of systolic and diastolic pressure shown. At 10 minutes 1.0 mg. of 1-hydrazinophthalazine (5968) was injected intravenously, resulting in an immediate fall of blood pressure. A subsequent injection of 2.0 units of pherentasin caused little response; however, 1.0 unit of angiotonin was pressor. At 35 minutes 5.0 mg. of 5968 was injected; the response to another unit of angiotonin 5 minutes later was not inhibited.

PHENOLIC AMINES. Unlike indolic amines, their phenolic counterparts were physiologically blocked by hydrazinophthalazine. The pressor activity of *phenylethylamine* was usually markedly reduced by 5 mg. of the antihypertensive agent. To inhibit *tyramine* and *nor-epinephrine* took somewhat larger amounts, and even so, doubling the amine broke through the inhibition. The diminution of the physiologic effect of *arterenone* (the β-carbonyl derivative of nor-epinephrine) was intermediate between phenylethylamine on the one hand and tyramine and nor-epinephrine on the other. In order to check the difference between phenolic and indolic amines, the 2 types of compounds were injected alternately into 8 animals; increasing doses of hydrazinophthalazine inactivated only the former.

OTHER PRESSOR SUBSTANCES. The pressor response to *isoamylamine* was abolished after large doses (60 mg. per kg.) of the hydrazine, but quadrupling the amount of amine caused a significant return of response. The vasoactivity of *angiotonin* was sometimes lessened but never completely abolished by prior

TABLE 2.—HYDRAZINOPHTHALAZINE BINDING AS DETERMINED BY INHIBITION OF NINHYDRIN COLOR*

Material	*Final Millimolarity*	*Optical Density*	*Control Optical Density*	*Optical Density Depression*
		AMINES		
Methylamine	1.0	1.320	1.300	<0.05 *i.e.*, none
n-Propylamine	1.0	1.125	1.165	
Isoamylamine	1.0	1.320	1.360	
Ethanolamine	1.0	1.175	1.220	
Tyramine & Tryptamine	1.0†	1.185	1.200	
Arterenone	1.0	1.252	1.232	
Angiotonin‡	2.5 units/cc.	1.233	1.195	
Glycine, glutamic acid, tyrosine, & arginine	2.0†	1.217	1.222	
Alanine	1.0	1.201	1.222	
Serine	2.0	1.243	1.222	
		MERCAPTANS		
Cystine	2.0	1.267	1.278	<0.05
Cysteine	1.0	0.902	1.201	0.299
Glutathione	4.0	1.310	1.278	<0.05
Reduced Glutathione	4.0	1.168	1.278	0.110
βMercaptopropionic acid	1.0	1.017	1.201	0.184
βMercaptopyruvic acid	1.0	0.172	1.201	1.029
Thioglycolic acid	2.0	0.201	1.180	0.979
Thiomalic acid	2.0	0.098	1.252	1.154
Thiomalic acid	0.20	0.602	1.252	0.650
2,3-Dimercaptopropanol (BAL)	2.0	0.187	1.252	1.065
Ethyl mercaptan	2.0	0.632	1.252	0.620
		CARBONYL GROUPS		
Glucose	10	1.183	1.179	<0.05
Lactic acid	10	1.130	1.179	<0.05
αKetoglutaric acid	1.0	1.218	1.278	0.060
Propionic acid	1.0	1.187	1.201	<0.05
Pyruvic acid	1.0	0.670	1.201	0.531
Acetaldehyde	2.0	0.182	1.180	0.998
		STEROIDS		
Calciferol	0.10	1.219	1.260	<0.05
Dehydrocorticosterone acetate	1.0	1.287		
Cholesterol	100	1.307		
Estrone	0.70	1.259		
Digitoxin	2.5	1.335	1.370	
		METALS		
Li^+, Na^+, K^+, Be^{2+}, Mg^{2+}, & Ca^{2+}	10†	1.215	1.185	<0.05
Zn^{2+}, Pb^{2+}	1.0†	1.155	1.160	
Cd^{2+}	1.0	1.378	1.370	
Al^{3+}, As^{5+}	1.0†	1.183	1.160	
V^{3+}	0.01	0.240	1.260	1.020
V^{3+}	1.0	0.940	1.260	0.320
V^{5+}	10	0.0 ± 0.025	1.315	complete
V^{5+}	1.0	0.860	1.315	0.455
Cr^{3+}	1.0	1.183	1.160	<0.05
Mn^{2+}	10	0.150	1.160	1.010
Mn^{2+}	1.0	0.848	1.160	0.312
Fe^{2+}	10	0.910	1.160	0.250
Fe^{2+}	1.0	1.037	1.160	0.123
Fe^{3+}	10	0.0 ± 0.025	1.160	complete
Fe^{3+}	1.0	0.215	1.160	0.945
Fe^{3+}	0.10	1.050	1.260	0.190
Co^{2+}	1.0	1.155	1.160	<0.05
Ni^{2+}	10	0.690	1.315	0.625
Cu^{2+}	1.0	0.0 ± 0.025	1.160	complete
Cu^{2+}	0.10	0.510	1.260	0.750
Ag^+	1.0	0.195	1.315	1.120
Sn^{2+}	10	0.0 ± 0.025	1.315	complete
Hg^{2+}	1.0	1.100	1.315	0.215

* 50 cc. of test material plus 0.50 cc. of approximately 0.01 molar hydrazinophthalazine plus 0.30 cc. 2% ninhydrin in isopropanol made to pH 3.0 with glacial acetic acid, heated for 7 min. at 70° C., cooled, extracted with an equal volume of chloroform, and read at 460 mμ. on the Beckman spectrophotometer.

† Concentration is with respect to each substance listed.

‡ Definite chemical combination with a solution of angiotonin supplied as the lyophilized solid was demonstrated in 2 experiments by depression of optical density to 0.376 for an approximately 2 mM. solution (on the basis of a molecular weight of 523). No combination could be demonstrated in more recent experiments using angiotonin supplied as a solution.

injection of the drug (Fig. 1). Heating the angiotonin to 70° C. at pH 3 for five minutes did not decrease its activity; but heating with the hydrazine at this pH rendered it less active. Pherentasin[5,16] was no longer active when given to rats pretreated with 1.0 mg. of intravenous hydrazinophthalazine (Fig. 1). The pressor activity of the *pervanadyl ion*[3], a prolonged pressor substance in the rat, similar in its vasoactivity to pherentasin, was inhibited *in vivo* by 5 mg. of the drug, perhaps by reduction of the metal. The effect of cadmium, a shorter acting substance, was also abolished. Of the pressor agents used, chemical evidence of *in vitro* interaction with hydrazinophthalazine was obtained only for angiotonin and pentavalent vanadium (Table 2).

The usual depressant action of intravenous hydrazinophthalazine on the normal rat failed to appear when the compound was mixed with pyruvate, but it was not diminished by incubation with cysteine. Much less than stoicometric amounts of ferric, cupric and pervanadyl ions failed to block the vasodepression of hydrazinophthalazine; however the first, at least, seemed to inhibit the typical nor-epinephrine response in the animals. Pyruvate was not similarly sympatholytic and cysteine only dubiously so.

Chemical Reactions. MERCAPTANS. *In vitro* interaction between therapeutic concentrations of hydrazinophthalazine and physiologic concentrations of sulfhydryl radicals, carbonyl groups, and certain metallic ions have been described[6,7]. Evidence of actual combination in body fluids has been obtained only for mercaptans, which were found bound to the drug in urine[6].* In another paper it is shown that the ratio of free drug to "bound" drug gradually rose as therapy continued[9]. An *in vitro* reaction was demonstrated between hydrazinophthalazine and the mercaptans tested. On the other hand, the disulfides seemed inert (Table 2). Beta sulfhydryl and alpha carbonyl derivatives of propionic acid reacted with hydrazinophthalazine; whereas alpha amino derivatives did not (Table 2).

CARBONYL COMPOUNDS. Since a reaction between hydrazinophthalazine and carbonyl groups might be manifested by a fall in the circulating pyruvate, plasma pyruvate concentrations were determined by the method of Friedman and Haugen[2]. The mean value was 1.26 with a standard deviation of 0.17 mg./100 cc. for 10 untreated hypertensive patients and 1.26 with a standard deviation of 0.19 mg./100 cc. for 10 patients on adequate doses of Hyphex. The absence of measurable change may have been due to the disparity between the concentration of pyruvate and that of hydrazinophthalazine in blood; the former concentration was of the order of 140 μM/L. and the latter did not ordinarily exceed 3 μM/L.[9]. Total carbonyl levels were also measured by the method of the same authors[2]. The averages for 6 untreated and for 6

*Binding was determined by measuring the inhibition of the ninhydrin-hydrazine color in the presence of the test material. A standard color was developed according to methods that will be described[9], and compared spectrophotometrically with the color obtained after incubation with the compound to be assayed. As a control, ninhydrin was allowed to react with amine in the presence of the test compound in order to exclude the possibility of the inhibition being mediated via ninhydrin rather than hydrazine. Combination with rather than destruction of the hydrazine was indicated by the reversibility of the process in the presence of heavy metals with a high affinity for sulfhydryl compounds. Since the entire expected opacity appeared in the aqueous medium used to develop the color, none of the test compounds had its effect by altering the water-chloroform partition coefficient of the complex. To disprove the possibility of an effect on the equilibrium of complex formation without actually combining with one of the reactants is more difficult.

treated persons were 3.81 and 3.93 mg. per 100 cc. of plasma respectively. *In vitro* interaction was demonstrated for pyruvate and acetaldehyde but not for glucose and probably not for α-ketoglutarate (Table 2).

No *in vitro* reaction could be shown between hydrazinophthalazine and steroid substances. Those tested included cholesterol and several containing carbonyl groups (Table 2).

METALS. Substituted hydrazine reacted with certain heavy metals. The alkali metals and the alkaline earths as well as zinc and several others proved inert. On the other hand, the elements with atomic numbers from 25 to 29, including the transition group, reacted to varying extents. The interaction was most marked with the cupric ion whereas it could not be proven with the cobaltous ion. Ferric and pervanadyl ions prevented all color formation by oxidizing the hydrazine with the evolution of gas.* Ferrous iron, in dilutions as low as 10 micromolar, gave a characteristic pink color with the drug, which was apparently due to formation of a complex ion that migrated to the cathode in an electric field.

Clinical Findings. Despite the lack of obvious interaction between hydrazinophthalazine and the entire cholesterol molecule, there was a rapid fall in total serum cholesterol after the inception of therapy; however, when treatment was maintained for 6 months or more the level tended to return to its initial value (Table 3). Within 30 days of starting the drug, all but 3 of 25 consecutive cases showed a decline in cholesterol; the mean change was 41 mg. per 100 cc. Despite the eventual rebound none of the 10 patients with more than 275 mg. per 100 cc. initially showed a return to within 25 mg. per 100 cc. of the control value.

In view of the action of phenylhydrazine, erythrocyte counts and hemoglobin determinations obtained immediately before therapy were compared with values found after 2 weeks to 2 years of treatment. Of 52 consecutive patients, 2 were excluded because they had less than 4 million red blood cells per c.mm. prior to taking hydrazinophthalazine. Among the remainder there was a tendency for moderate anemia to develop. The effect was maximal after 1 to 3 months of continuous drug ingestion; thereafter it continually lessened, becoming negligible after a year (Table 3). The anemia was apparently normocytic and normochromic since the average diminution of 1.41 gm. per 100 cc. of hemoglobin corresponded to a mean decrease in erythrocytes of 0.446 million per c.mm. giving a mean corpuscular hemoglobin in the vanished cells of 31.6 γγ.

Because of affinity between hydrazinophthalazine and the transition group of elements, iron, copper, and cobalt were fed to hypertensive patients. Patients who had been on Hyphex for a year or more and whose blood pressures and medication were stabilized made a sensitive test system for antihypertensive agents. A potent drug altered their need for hexamethonium ion and they automatically changed their intake in an attempt to maintain their previous blood pressure level. Preliminary clinical observations on 6 patients so stabilized suggested that a daily supplement of 50 mg. of cobalt chloride and 50 mg. of pyridoxal decreased the hexamethonium re-

*An excess of ferric ion liberated 0.84 mg. atoms of nitrogen (9.4 cc. of N_2 corrected to standard temperature and pressure) per millimole of hydrazinophthalazine. This suggested oxidation of the terminal nitrogen atom of the hydrazine with concomitant reduction of the iron to the ferrous state. Pervanadyl ion liberated 1.58 mg. atoms of nitrogen (17.7 cc. of N_2 corrected to standard temperature and pressure) per millimole of drug, apparently oxidizing both nitrogen atoms of the hydrazine.

TABLE 3.—DIMINUTION OF CIRCULATING CHOLESTEROL AND HEMOGLOBIN IN 50 PATIENTS* DURING HYPHEX THERAPY

PART 1. CHOLESTEROL

Days after Beginning 1-hydrazinophthalazine

	0 to 30	*31 to 90*	*91 to 180*	*181 to 365*	*over 365*	*Total*
Number of cases†	21	5	13	6	5	50
Mean diminution (mg./100 cc. in serum)	40	60	50	1	0	36
No. consistent	18	5	11			
Number of cases†		39		11		
Mean diminution (mg./100 cc. in serum)		46		1		

PART 2. HEMOGLOBIN

Number of cases‡	2	10	16	12	10	50
Mean diminution (gm./100 cc. in blood)	1.15	2.02	1.55	1.37	0.69	1.41
No. consistent	2	10	14	11	6	

* The first 50 consecutive instances for which the data were available.

† 2 cases in which the control plasma cholesterol values were 106 and 123 mg. per 100 cc. were excluded; no other values were less than 170 mg. per 100 cc. and there were no other exclusions.

‡ 2 cases in which the control erythrocyte values were less than 4 million per c.mm. were excluded; there were no other exclusions.

TABLE 4.—EFFECT OF ORAL COBALT ON THE BLOOD PRESSURE AND HEXAMETHONIUM REQUIREMENT OF HYPERTENSIVE PATIENTS TREATED WITH HYPHEX

	Before Cobalt		*During Daily Administration of 50 to 60 mg. Cobalt*				
			Days for Which B.P. and Dose are Averaged (Numbered from Start of Cobalt)			*% Decrease*	
	B.P.	*mg. C_6/day*		*B.P.*	*mg. C_6/day*	*B.P.*	*C_6/day*
M. D.*	$144/93^{42}$	3.12^{42}	22–42	$139/91^{21}$	0.90^{21}	3/neg.	72
E. M.*	$133/84^{35}$	1.94^{35}	22–42	$132/81^{21}$	1.84^{21}	neg./4	5
J. K.*	$180/103^{28}$	1.86^{28}	22–28	$171/106^{7}$	1.60^{7}	11/neg.	14
J. L.*	$160/99^{28}$	2.29^{28}	22–28	$161/101^{7}$	1.96^{7}	neg./neg.	14
L.H.*	$157/82^{30}$	1.44	43–56	$155/78^{14}$	0.88^{14}	neg./5	39
M. B.*	$166/95^{28}$	0.93^{28}	8–14	$157/91^{7}$	0.79^{7}	5/4	15
B. B.**	$142/86^{7}$	2.41^{7}	3–10	$131/78^{7}$	1.09^{7}	8/9	55
H. D.†	$161/102^{3}$	1.00^{3}	11–14	$114/82^{3}$	0.08^{3}	29/20	92
M. C.‡	$232/139^{5}$		60–65	$153/92^{5}$		34/34	

Superscripts after blood pressure values and hexamethonium dosages indicate the number of days for which pressure readings or medication were averaged. The pressure was determined and the drug taken five times each day. "Neg." in the seventh column indicates a change of less than 3 %.

* Each patient had received Hyphex for at least one year. This was sufficiently long to produce a constant mean blood pressure with a constant average dose of both drugs. The month or 6 weeks immediately before cobalt therapy was used as the control (Before Cobalt). During this time a daily "vitamin capsule" containing 50 mg. of pyridoxal was given; no significant change in pressures or drug requirement was noted. At the end of this period an additional daily "vitamin capsule" containing 50 mg. of cobalt chloride was begun. Otherwise nothing was altered. No symptomatic changes occurred. The hydrazinophthalazine intake remained the same.

** B. B. began Hyphex 3 weeks before cobalt. During both the control week and the 10 days of cobalt therapy, the hydrazinophthalazine dosage remained 0.45 gm./day.

† H. B. was rehospitalized for weakness 3 days prior to the onset of cobalt administration. Her hydrazinophthalazine dosage remained 0.6 gm./day until discharge. Hyphex had been started 9 months previously.

‡ Because collagen disease was suspected, M. C. stopped Hyphex 4 months before beginning cobalt. During the 5-day control period no medication was taken. She took 50 mg. of cobalt chloride a day for 28 days and then stopped. Because there was no hypotensive effect she took 40 gm. of hexamethonium chloride during the next 22 days. Whereupon her blood pressure fell so that for 10 days she took no drugs of any kind. During the next 5-day period which, for convenience, is listed in the table under "During Cobalt" she continued to take no drugs of any kind. Her pressure during this interval is the more remarkable since between 3 and 5 gm. per day of hexamethonium chloride alone had not kept her systolic blood pressure below 200 mm. Hg 2 months before metal therapy.

quirement by 25% (Table 4). Similar data are tabulated for 3 other patients where the effects of cobalt were more striking but where other variables may have affected the results.*

Discussion. The widely used drug, 1-hydrazinophthalazine, proved to be highly reactive *in vitro*. It had no affinity for the common alkaline earths, alkali metals, or zinc, but it reacted with iron, manganese, and copper which are necessary for certain enzymatic processes. Furthermore, the hydrazine group was a carbonyl reagent. In addition it reversibly bound aliphatic mercaptans, perhaps as organic sulfides.

Unfortunately little is known of the pharmacology of hydrazinophthalazine other than that it is a vasodilator of unique action, especially involving blood flow through the kidneys[18]. In the rat, at least, the pressor action of the primary amines that would result from decarboxylation of naturally occurring amino acids is diminished only by amounts of drug far in excess of therapeutic levels.† If there is such a phenomenon as "nonspecific" inactivation, all of the organic compounds tested, with 2 exceptions, could be considered to be so inactivated. One of these exceptions is pherentasin obtained from extracts of hypertensive blood, which became completely inert after "therapeutic" doses of the hydrazine. Elevated blood pressure fell after injection of the drug or failed to rise when both the drug and the pressor material were given together. Pherentasin is believed to contain a carbonyl group; whether or not direct chemical combination occurs is not known. The other exception is indolic amine, either hydroxylated or not, the pressor activity of which was enhanced. We cannot explain the discrepancy between the reported inactivation of serotonin (5-hydroxytryptamine) by hydrazinophthalazine in the dog[17] and our results in rats other than on the basis of species specificity.

The mild anemia which almost always followed the administration of substituted hydrazine may be related to loss of iron or copper for which this agent has a strong affinity. This degree of anemia has not been observed in patients taking hexamethonium ion alone. That it regressed despite the continuance of therapy suggests that drug sensitivity was not involved. The fall in total cholesterol is unexplained; it is presumably not due to direct chemical combination between the drug and the entire cholesterol molecule. Studies of liver function in 50 patients failed to disclose any evidence of hepatocellular damage.

The nephrogenic component of hypertension is blocked by a varied array of substances whose only obvious common characteristic is an affinity for certain metal ions. These are hydrazinophthalazine, thiocyanate, tetrasodium pyrophosphate[13], dimercaptopropanol and other aliphatic mercaptans[12], and common metal chelators such as ethylenediamine tetraacetate and 8-hydroxyquinoline[13]. It is possible that they act by removing a metallic coenzyme in the kidney or blood vessels. The effect of cobalt is uncertain but it

*Cobalt had an unusual vasodepressant effect on renal hypertensive rats. All of 4 such animals with diastolic pressures over 100 mm. Hg showed a significant lowering of the diastolic pressure with a mean fall of 47 mm. Hg about 40 minutes after the intravenous injection of 0.6 mg. cobalt chloride. The mean change for 3 normal normotensive rats after a similar dose was 2 mm. Hg with one animal showing a rise, one a fall, and one no change.

†Therapeutic parenteral doses in human malignant hypertension were found to be approximately 20 to 40 mg. per hour or roughly 0.3 to 0.6 mg. per kg. per hour. Adequate oral doses have ranged from 100 to 200 mg. every 4 hours. In the rat, 1.0 mg. approximates 4.0 mg. per kg.

is conceivable that an excess may tend to displace another metal by mass action.

Summary and Conclusions. The chemical properties of 1-hydrazinophthalazine were investigated *in vitro*. This drug had an affinity for certain trace metals, some carbonyl compounds, and the aliphatic mercaptans tested. Prolonged use in man caused temporary mild anemia and a fall in the total serum cholesterol content. Its pharmacology in rats was studied. Blockade of the pressor action of "natural" primary amines was not achieved until very large doses were given. Pherentasin was inactivated by small doses.

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STUDIES ON THE CONTROL OF HYPERTENSION BY HYPHEX. IV. LEVELS OF THE AGENTS IN URINE AND BLOOD

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Because therapy of severe arterial hypertension with oral hexamethonium chloride and 1-hydrazinophthalazine (Hyphex)† is highly effective[17,20], therapeutic levels of these agents in blood and their rate of renal excretion were studied. Methods for determination of the hexamethonium ion[25] and the hydrazine[12] have been reported.

Methods. HEXAMETHONIUM ION. Hexamethonium assay was accomplished essentially by the technique of Zamis[25] and depended on the formation of a colored reineckate which was soluble in acetone but not in water. The procedure used for urine follows: 10 cc. of a saturated aqueous solution of ammonium reineckate, $NH_4[Cr(NH_3)_2(SCN)_4]$, was added to a 100 cc. aliquot of a 24-hour specimen. The mixture was left at room temperature for 30 minutes and then filtered with suction through fine sintered glass in the presence of about 3 cc. of *Celite* analytical filter aid. The clear red filtrate was discarded, and the precipitate was washed several times with 25 cc. portions of water until the washings were colorless. Fifteen cubic centimeters of acetone were mixed with the precipitate and allowed to stand for 5 minutes before filtering. This was repeated until the acetone too was colorless. The total volume of the pooled acetone was noted, and its optical density at 525 millimicrons (mμ) was measured on the Beckman spectrophotometer. The hexamethonium concentration was found by comparison with a standard curve. To check recovery hexamethonium halide was added to 10 different urines to the extent of 20 mg. of cation per 100 cc. On assay the standard deviation from the theoretical value was 2.2%; 2 mg. of hexamethonium was necessary for adequate estimation. With the technique outlined no acetone color was obtained when urines from 20 untreated patients were used as blanks. Moreover once a urine containing hexamethonium ion was treated with reineckate and filtered, a second addition of this reagent failed to produce more crystals. Saturated ammonium reineckate was added to solutions of several amines and amino acids which may occur in low concentrations in human urine. No precipitation occurred with 0.01 molar glycine, leucine, histidine, methylamine, or histamine; whereas a faint cloud formed with isoamylamine and a very marked crystal sheen with trimethylamine. Dilution

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†Hyphex refers to the combination of these 2 drugs used according to the method described[17,20]. 1-Hydrazinophthalazine (hydralazine) is available as "Apresoline" (Ciba).

of these two amines to 0.001 molar prevented any visible reaction.

The concentration in blood was approximated as follows: One-half gram of solid trichloracetic acid was added to 5 cc. of plasma or serum. The mixture was permitted to stand for at least 6 hours before centrifugation in order to insure a completely clear supernatant. Two cubic centimeters of the deproteinated blood was then added to 0.2 cc. of saturated aqueous ammonium reineckate. The resultant turbidity was compared with that of simultaneously prepared standards. Because of the tendency for amorphous rather than crystalline precipitation in body fluids the most accurate standards were made up in plasma. Adding 0.05 mg. of the ion to 10 cc. of drug-free plasma indicated that the expected error was 20% if the comparison was made visually. Errors of this magnitude were of no clinical significance in determining the presence or absence of acute intoxication; however, they could be reduced with a nephelometer. Neither normal blood nor aqueous solutions of amines and amino acids in roughly physiologic concentrations gave turbidity under the conditions outlined.

HYDRAZINOPHTHALAZINE. Quantitation of 1-hydrazinophthalazine depended on the colorimetric assay of its hydrophobic ninhydrin complex after extraction with chloroform. A detailed method for urine was developed accurate to better than 3% at concentrations of 200γ per 100 cc. or more. Estimation of the drug level in plasma depended on the same procedure, but was less accurate because of limited volume, lower concentration, and interfering substances. Therefore the highest ninhydrin concentration giving negative blanks and the maximal practical plasma volume were used in conjunction with the minimum amount of chlorofrom. In obtaining the values cited later, 0.20 cc. of 2.0% ninhydrin in isopropanol was added to 10 cc. of a protein-free filtrate of blood prepared by adding 10% solid trichloracetic acid to plasma and centrifuging. The ninhydrin-hydrazinophthalazine complex was then extracted into 0.50 cc. of chloroform. When the assay was checked by adding therapeutic concentrations of the drug to normal plasma the error approximated 25%. Mensuration of 1,4-dihydrazinophthalazine was identical to that of the monohydrazine save that the molecular extinction coefficient was higher and the maximum optical density occurred at 480 rather than 460 mμ[12].

Cysteine, reduced glutathione, and other water soluble mercaptans inhibited the formation of a colored product when hydrazinophthalazine was incubated with ninhydrin according to the procedure previously described for urine[12].* Possibly the hydrazine and mercaptan formed a salt, a substituted sulfide. If certain heavy metals with an affinity for sulfyhdryl groups were added to the system, this inhibition was partially blocked. It was discovered that the ninhydrin color obtainable in the urine of patients receiving hydrazinophthalazine was enhanced by the careful addition of an antimony solution. To determine whether the amount or proportion of "bound" drug varied systematically, the following approximate technique was evolved to measure the maximum increment of ninhydrin-hydrazine color. Into 100 cc. aliquots of urine were pipetted successively doubled amounts (from 0.1 to 1.6 cc.) of 40% antimony trichloride in 10% aqueous hydrochloric acid. After 15 minutes 0.50 cc. of 2.4% ninhydrin in isopropanol was added to each solution and the pH was adjusted to 3.0 ± 0.2 with saturated sodium hydroxide. Thereafter the samples were heated, cooled, and extracted with chloroform in the same manner as routine hydrazinophthalazine determinations[12]. The difference between the optical density found without antimony and the highest value in its presence was an index of the drug associated with mercaptans. By careful titration with antimony trichloride it was possible to recover 80% of the ninhydrin color. It was necessary to titrate the antimony since an excess inhibited the yellow of the ninhydrin complex and so decreased the final color; whereas a dearth had a similar effect as a result of failure to release the maximum amount of hydrazinophthalazine.

Hexamethonium Values. RENAL EXCRETION AFTER ONE ORAL DOSE OF HEXAMETHONIUM CHLORIDE. The excretion pattern of ingested hexamethonium ion was notably variable, presumably because quaternary ammonium com-

*This reaction was sufficiently reproducible to allow the estimation of urinary sulfhydryl concentrations, that is, millimolar cysteine halved the ninhydrin color of 0.1 mM. hydrazinophthalazine. The decreased optical density was present in the aqueous solution prior to extraction with chloroform and therefore was due to diminution of complex formation rather than failure of extraction. An apparently normal ninhydrin-amine reaction in the presence of mercaptan suggested that the mercaptan combined with the hydrazine rather than with the ninhydrin.

pounds are incompletely absorbed from the gastrointestinal tract[4,5]. Figure 1 indicates that 15% of a single massive oral dose was excreted in the urine. Half of this appeared within 10 hours, and two-thirds within one day, although measurable levels persisted for 5 days. Urinary recovery after a single average of 36% appeared within 4 hours, 68% within 8 hours, and 82% within 12 hours.

RENAL EXCRETION AFTER CONTINUOUS ORAL HEXAMETHONIUM CHLORIDE. Over 300 analyses were performed. Figures 2 and 3 demonstrate that for persons on Hyphex treatment renal excretion of

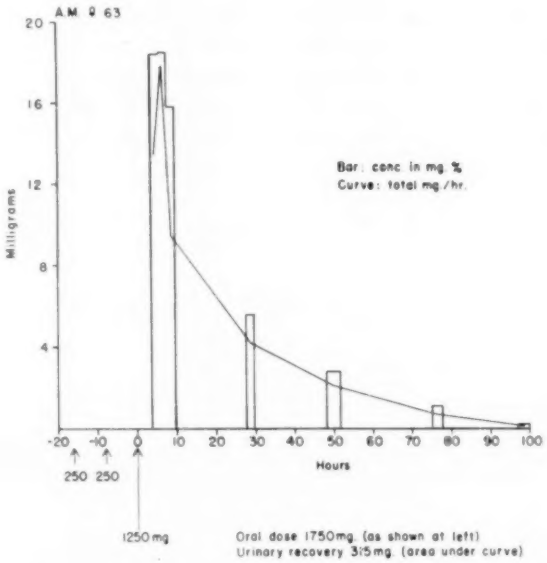

Fig. 1.—Excretion of a single massive oral dose of hexamethonium chloride. The bar graph indicates urinary concentrations in mg. per 100 cc. The curve shows the hourly output. Of 1750 mg. ingested approximately 315 (the area under the curve) were excreted by the kidney.

A.M. was a 63-year-old white female with angina pectoris, signs of calcific aortic stenosis, and a history of hypertension for 3 decades. During the year prior to this hospital admission her systolic pressure had been lowered from approximately 250 to 180 mm. Hg by 0.25 gm. of oral hexamethonium chloride 5 times a day. One month before entry she began to develop abdominal signs which culminated in the removal of a strangulated ovarian cyst. Three days after surgery she was started on 0.25 gm. of oral hexamethonium chloride every 8 hours, after being without antihypertensive medication for several weeks. Through an error, the third dose was 5 times that prescribed. Despite this excessive amount her blood pressure never fell below 140/80 mm., and there were no untoward symptoms.

smaller oral dose ordinarily exceeded 5%, but both the time and the amount of excretion varied within wide limits. In 8 cases after 250 mg. by mouth the mean percentage recovered in 24 hours was 5.8, the maximum 10.1 and the minimum 2.8. Of that recovered an the agent was correlated with oral intake rather than therapeutic result. In 6 patients whose urine was quantitatively investigated continuously during 3 to 5 weeks of hospitalization, the mean recovery of ingested hexamethonium chloride was 3.5% with extremes of

7.1 and 2.9%. The maximum urine concentration was 150 mg. per 100 cc., found in a 3-hour specimen from a man who was receiving 5 gm. a day. At the end of the collection period there was 0.5 mg. per 100 cc. in his plasma.

RENAL EXCRETION AFTER CONTINUOUS PARENTERAL HEXAMETHONIUM BROMIDE. Clinically, parenteral medication was at least 10 times as effective as oral medication and the percentage recovery of the drug was increased about 10-fold suggesting that only a small fraction of the material was absorbed from the gastrointestinal tract. For instance, in the patient described in Fig. 6 a mean of 17.0 mg. per hour injected intramuscularly over a 3-day period approximated the hypotensive effect of a mean dose of 183 mg. per hour ingested orally during a similar interval. The average urinary recovery of the parenterally administered drug was 11.5 mg. per hour and that of the drug taken by mouth was 10.1 mg. per hour.

PLASMA LEVELS AFTER CONTINUOUS ORAL HEXAMETHONIUM CHLORIDE. The average plasma level of hexamethonium ion in 50 adequately treated patients was 0.37 mg. per 100 cc. One-third of

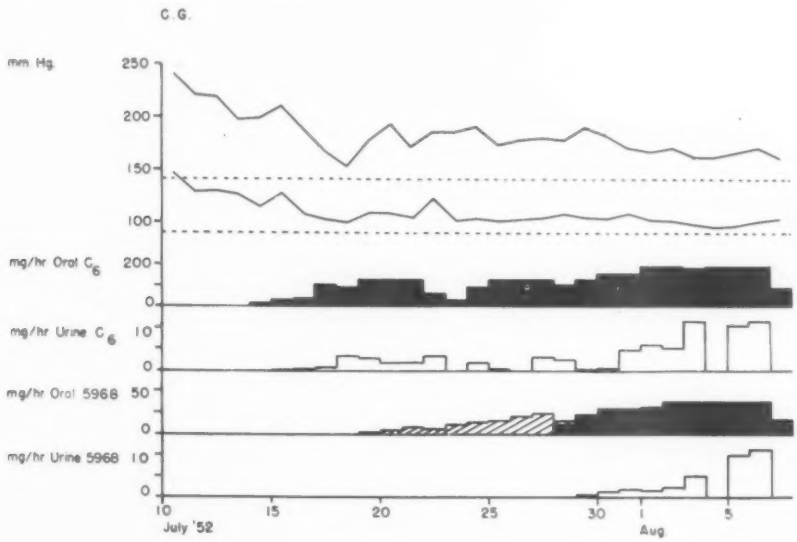

Fig. 2.—Oral dosage and urinary excretion of hexamethonium chloride (C_6) and hydrazinophthalazine (5968) with the supine blood pressure response (each point represents the mean of 6 measurements) indicated on the same time axis. Cross-hatching indicates 1,4-dihydrazinophthalazine. On 4 days (23 and 27 July, 4 and 7 August) the urine could not be obtained.

C.G. was a 47-year-old white female who entered the hospital with a blood pressure of 240/150 mm. Hg, cardiomegaly, and grade III retinopathy (Keith-Wagener). There was a history of hypertension for 5 years. An enlarged, abscessed kidney had been removed 30 years previously, and the remaining one had become infected 15 years later. On admission 3-plus albumin was present, but renal function was adequate. On bed rest alone her blood pressure fell to 200/130 mm. Hypex therapy was then instituted as shown.

In the first week after discharge from the hospital the mean blood pressure (35 determinations) was 157/98 mm., and her average oral daily medication was 3.75 gm. of hexamethonium chloride and 0.75 gm. of hydrazinophthalazine in divided doses. During the week exactly 6 months after discharge the mean blood pressure (34 determinations) was 153/93 mm. and her average daily medication was 2.66 gm. of hexamethonium chloride and 0.88 gm. of hydrazinophthalazine.

the concentrations were less than 0.1 mg.; another one-third were between 0.1 and 0.5 mg.; only one-tenth exceeded 1 mg., and none was greater than 1.5 mg. per 100 cc. There was a correlation between the amount ingested and the amount in blood. The mean oral daily dosage corresponding to the 3 ranges of plasma concentration were 2.3, 2.7, and 3.1 gm. respectively. These plasma concentrations are in excellent agreement with those found by bioassay after parenteral medication in hypertensive[8] and normal[9] subjects as well as after oral or injected drug in postpartum women and their newborn children[7].

In the presence of azotemia therapeutic blood concentrations were achieved with one-half to one-eighth of the usual doses[4,18]. Two patients with hypertension and nitrogen retention developed the autonomic paralysis and hypotension of acute hexamethonium overdosage[10]. A constipated uremic Negro woman had 12 mg. of drug per 100 cc. of plasma on admission to the hospital. After 48 hours without medication or bowel movement the level had risen to 16 mg. per 100 cc. Four

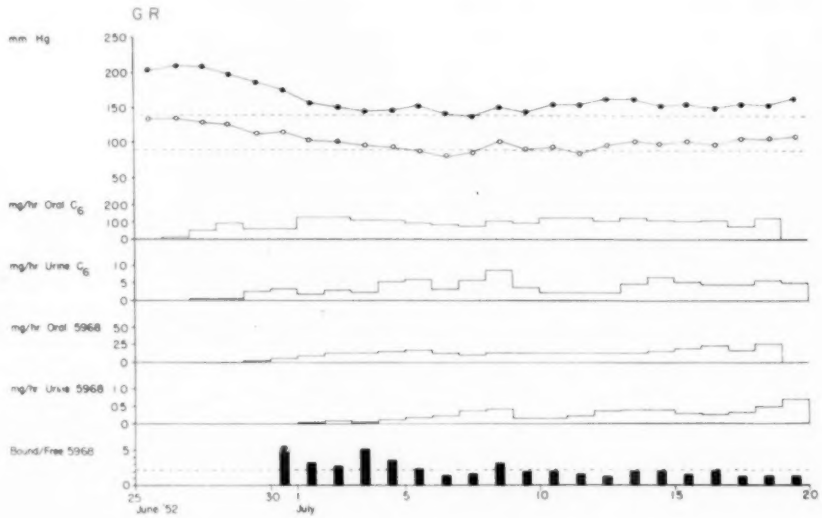

Fig. 3.—This 38-year-old white male entered the hospital with known albuminuria for 10 years and hypertension for 2. His blood pressure on admission was 210/140 mm. Hg. Fundoscopic examination revealed hemorrhages, exudates, and papilledema. There was cardiomegaly. There were only 2.5 million red blood cells per c. mm. of blood. The nonprotein nitrogen in the plasma was 47 mg. per 100 cc. (upper limit of normal 25 mg. per 100 cc.) and the excretion of intravenously injected phenolsulfonphthalein was 11% in 2 hours.

Ingestion and excretion of hexamethonium ion (C_6) and of free hydrazinophthalazine (5968) are indicated. As therapy progressed there was a decrease in the proportion of urinary hydrazinophthalazine "bound" to sulfhydryl compound. The dotted line at 2.3 is an arbitrary limit to which the ratio of "bound" to free drug never fell in the first 7 days of therapy and to which it never rose after the 10th day of therapy.

After discharge his blood pressure and clinical condition remained unchanged and he continued to work. Because of renal insufficiency no attempt was made to lower the blood pressure below 180/110. Four months after leaving the hospital he stopped his medication because "he ran out over a weekend." Within 48 hours he was dead of a massive cerebral hemorrhage.

days after the gastrointestinal tract had been emptied by laxatives and enemata the concentration was 5 mg. per 100 cc., and in another week it was within the desirable therapeutic range at 1 mg. per 100 cc. Signs and symptoms had disappeared by the time the level reached 5 mg. per 100 cc. Another very similar constipated azotemic Negro woman had 8 mg. of hexamethonium ion per 100 cc. of plasma. Within 24 hours without drug the concentration had risen to 10 mg. but 4 days later it was 0.5 mg. per 100 cc.

In contradistinction to acute hexamethonium overdosage chronic toxicity was not characterized by an abnormally high concentration of drug in the blood. The presenting symptom was tachypnea which lessened when the patient was supine; this curious phenomenon has been noticed both by us[10] and by others[8]. The pathologic lesion was pulmonary interstitial fibrosis[16]. This syndrome has a predilection for colored men with malignant hypertension and nitrogen retention. We have never observed it in the absence of malignant hypertension. In 4 of 5 cases the patients were male, in 4 Negro, and in 3 azotemic.

Values of Substituted Hydrazine. RENAL EXCRETION AFTER ONE ORAL DOSE OF SUBSTITUTED HYDRAZINE. With the method used about 3% of a single oral 50 mg. dose of hydrazinophthalazine was excreted in the urine. In 8 instances the actual mean recovery was 2.9% with extremes of 1.0 and 4.4%. Half of the recovered amount appeared within 2½ to 5 hours and the rest within 24 hours[12]. For an untreated hypertensive patient 50 mg. orally sometimes acutely reduced the systolic pressure 30 or 40 mm. Hg with concomitant nausea, vertigo, and asthenia. The effect was maximal from 1 to 3 hours after medication.

There was a similar clinical effect after a comparable dose of 1,4-dihydrazinophthalazine; however, this compound could never be definitely demonstrated in urine. A substance spectrophotometrically similar to substituted hydrazine was usually present, but on the basis of absorption spectrum no more than 10% of it could have been unaltered dihydrazine. Urinary excretion of this material was much less than for 1-hydrazinophthalazine (assuming a molecular extinction coefficient of 1.37×10^4); recovery never exceeded 0.5%.

RENAL EXCRETION AFTER CONTINUOUS ORAL SUBSTITUTED HYDRAZINE. Over 300 analyses were performed. Typical renal excretion of free drug is indicated in Figs. 2 and 3. Urinary recovery of free hydrazinophthalazine in 6 orally treated patients studied in the hospital for 3 to 5 weeks varied from 2.0 to 0.3% with a mean of 1.1%. The corresponding extreme values for "total" (free + "sulfhydryl-bound") drug were 6.6 and 0.4 with an average of 2.7%. In Figs. 5 and 6 "total" renal excretion of hydrazinophthalazine is shown simultaneously with the free drug. The maximal concentration found in urine was 6 mg. per 100 cc. with half "bound" to mercaptan.

The cross-hatched part of the hydrazinophthalazine (5968) dosage in Fig. 2 represents 1,4-dihydrazinophthalazine. In this particular patient there was no measurable output of the drug for the 10 days during which the dihydrazine was taken; however, excretion was prompt after monohydrazine was substituted. Ordinarily the latter proved the more potent drug. In Fig. 2, however, this effect is not obvious.

Part of the urinary hydrazinophthalazine always occurred in "bound" form, presumably associated with mercaptan. As therapy progressed the free fraction tended to increase, although there was considerable variability in response (Fig. 3). More than 100 determinations

of the urinary ratio of "bound" to free drug are summarized in Fig. 4. For the first and second days of treatment the mean ratio was 2.22; for the third and fourth days it was 2.04; for the next pair of days it was 1.45; and for the next 1.31. The average for the interval from 11 to 15 days was 0.98 and from 16 to 20 days was 0.88. Individuals who needed only small doses of largely been free, all of it appeared "bound" on the following day. His blood pressure fell to 160/90 mm. Hg; whereas the lowest systolic reading in the previous week (42 determinations) had been 180 mm. (Fig. 5).

RENAL EXCRETION AFTER CONTINUOUS PARENTERAL SUBSTITUTED HYDRAZINE. Several times parenteral hydrazinophthalazine was given in small amounts

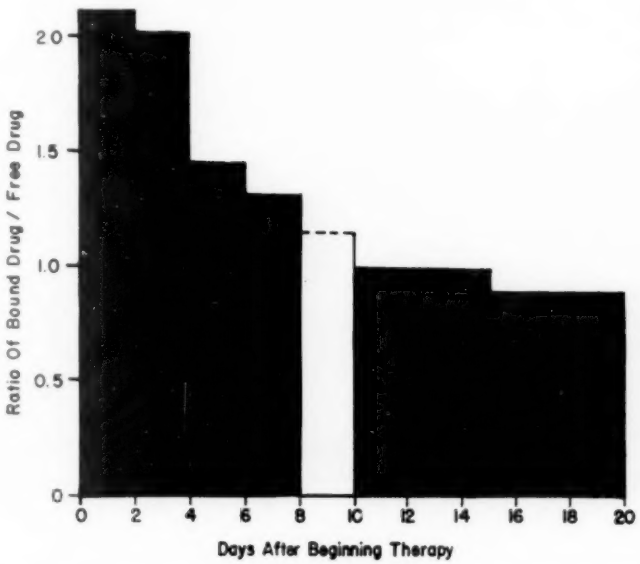

Fig. 4.—A total of 125 urine specimens were collected from 10 patients at varying times after initiation of hydrazinophthalazine therapy. On each specimen a determination of the ratio of "sulfhydryl-bound" drug divided by free drug was made. These quotients were grouped according to the prior period of hydrazine treatment. The means for 2- and 5-day periods are plotted above. For the interval from 8 to 10 days there was insufficient data.

drug for mild hypertension had atypical progression of their ratios.

Two hundred milligrams of dimercaptopropanol (BAL, British antilewisite), which has been variously reported as depressor[8,19] and pressor[1] in hypertensive persons, were given intramuscularly over a 12-hour period to a patient who had been on Hyphex therapy for one month and who was not well controlled. Although previously urinary hydrazinophthalazine had over relatively short periods without much success. The solid part of the hydrazinophthalazine dosage in Fig. 6 represents one such trial. Five milligrams per hour were given intramuscularly for 6 days. The excretion was low and the effect on blood pressure was slight; both increased with larger oral doses. When given parenterally the drug was not appreciably more effective as a vasodepressant than when given orally; moreover, urinary excretion

was independent of the route of administration. Both observations suggested that absorption from the gastrointestinal tract was good.

PLASMA LEVELS AFTER CONTINUOUS ORAL SUBSTITUTED HYDRAZINE. Plasma levels in 10 adequately treated patients ranged from 0.06 to less than 0.005 mg. with a mean of 0.023 mg. per 100 cc. Higher values were found in uremic patients, the highest being 0.40 mg. per 100 cc. One Negro woman with malig-

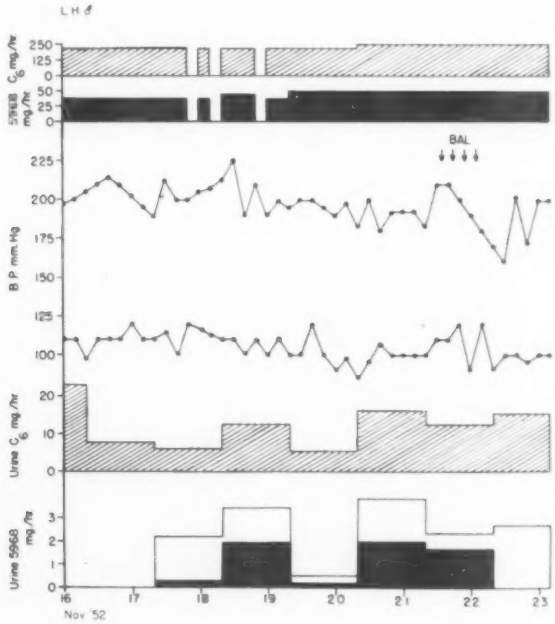

Fig. 5.—This 59-year-old coal miner came to Barnes Hospital with a 10-year history of hypertension and kidney trouble. Nine months prior to entry he was hospitalized elsewhere for a stroke. At this time he was told he had chronic Bright's disease and heart trouble for which digitalis was begun. His condition deteriorated progressively and he noted asthenia, anorexia, amblyopia, orthopnea, dyspnea, edema, and weight loss of 25 pounds. The physical examination on admission revealed a blood pressure of 220/140 mm. Hg, fundal hemorrhages and exudates, papilledema, dyspnea and orthopnea, basal râles, cardiomegaly, hepatomegaly, and 4-plus pedal edema. His urine showed casts and red cells as well as 4-plus albumin; however, the nonprotein nitrogen content of his blood was normal. The mean blood pressure was lowered to 190/110 mm. with 200 mg. of hydrazinophthalazine and 1000 mg. of hexamethonium chloride orally every 4 hours. On November 21, the systolic pressure was further lowered by intramuscular dimercaptopropanol (BAL) to 160 mm. which was 20 mm. below the previous minimum. Four 50-mg. doses of this drug were given at 4-hour intervals as shown by the arrows; local irritation prevented continuation of this therapy. Medication is shown above the blood pressure and urine levels below it. Cross-hatching indicates hexamethonium (C_6) ingestion and excretion. Solid black indicates intake and output of free hydrazinophthalazine (5968); the enclosed white area represents "bound" hydrazinophthalazine in the urine. (No values for urinary hydrazinophthalazine are available for November 16.)

A year after his hospital discharge this patient was asymptomatic without digitalis. His average blood pressure for the most recent month (March, 1954, with 120 readings) of available follow-up was 157/81 with a daily mean drug dosage of 1.64 gm. of hexamethonium chloride and 0.6 gm. of hydrazinophthalazine.

nant hypertension, who was receiving no hexamethonium salt but was ingesting 2.4 gm. of hydrazinophthalazine a day, had an apparent concentration of 1.0 mg. per 100 cc.

After continuous Hyphex therapy for 2 to 30 months 8% of patients noted manifestations of toxicity from hydrazinophthalazine[10,13]. Plasma concentrations of both drugs in such patients tended to be low probably because only small doses were needed to control the blood pressure.

Tolerance to Hyphex has not been observed. Thirty consecutive patients, most of them in severe stages, on whom we have continuous daily records for a year, were able to maintain their immediate post-hospital blood pressure levels without significant alteration in their dosage schedule (Table 1). The required doses for 10 of these patients who have continued this regimen for 2 years tended to decrease (Table 1). In all 30 instances the routine involved taking blood pressure in the seated position 5 times a day in order to gauge each dose of hexamethonium chloride needed[17,20]. Each value in the table is an average of the means for every patient. Each patient's mean, in turn, is composed of all the values for an entire week and usually was compiled from 35 blood pressure readings and 35

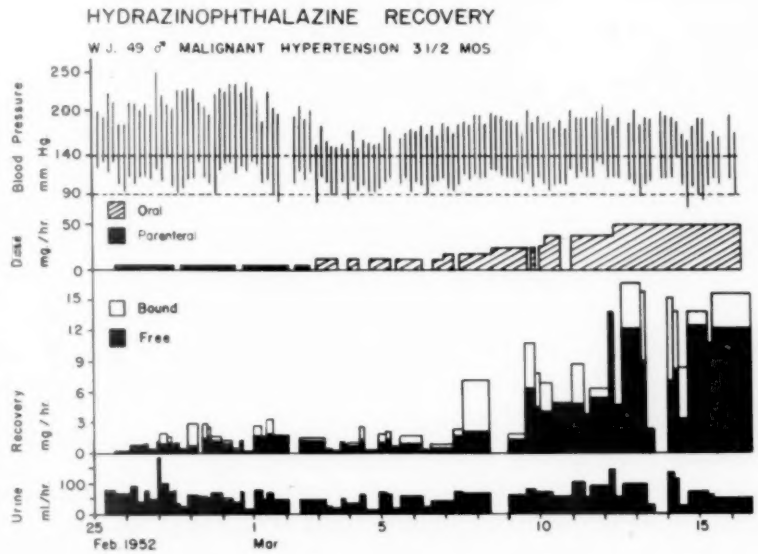

Fig. 6.—Oral and parenteral dosage of hydrazinophthalazine and supine blood pressure are plotted on the same time axis as urine volume and renal excretion of both free and "bound" drug. Urine samples were lost on 2, 8, and 13 March.

W. J. was a 49-year-old Negro male with fulminating malignant hypertension of less than 3-months' duration. He entered the hospital in February, 1952, with a blood pressure of 230/150 mm. Hg. Hexamethonium therapy was begun on 14 February. The average blood pressure had fallen to 200/130 by the time hydrazinophthalazine was begun on 25 February. In a month his blood pressure was 175/100 mm. and he went home symptomatically improved, taking 5 gm. of hexamethonium chloride and 1 gm. of hydrazinophthalazine daily in divided doses. He later expired and on postmortem examination was found to have arteriolar nephrosclerosis and interstitial pneumonia[16].

doses of both antihypertensive agents.

Discussion. Urinary excretion of hexamethonium salts gave no obvious indication of the efficacy of therapy. Regardless of hypotensive effect, barring shock, the amount put out by the kidneys depended largely on intake and renal function. It is our strong clinical impression that no severely hypertensive patient initially obtained sustained normotension with Hyphex unless hexamethonium chloride was given every 4 hours; this is not surprising in view of the rapid urinary excretion and relatively short action[4,11,21]. There was no evidence for the *in vivo* breakdown of the ion. Our inability to recover more than two-thirds of a parenteral dose was compatible with incomplete collections since this method of administration was used only in comatose or otherwise uncooperative patients who were at least partially incontinent. Others have recovered 60 to 85% in animals[23,25] and as much as 100% in man[2,4,5,23].

Hydrazinophthalazine proved highly reactive chemically; however, its mode of action is unknown. Proof of central nervous effect is indirect[3,22]; the drug injected intra-arterially in man dilates only the homolateral area[24]. The renal vasodilatation in the face of a lowered blood pressure is striking[15,24]. More than 90% of a dose was altered chemically, perhaps by metal ions or carbonyl compounds. Small fragments, free phthalazine, metallic complexes, or conjugated products such as hydrazones may have resulted. The frequent anemia may have been related to the affinity of hydrazinophthalazine for

TABLE 1.—MEAN DOSAGE AND BLOOD PRESSURE

	In hospital before Treatment	*Immediately after Discharge*	*1 Month after Discharge*	*1 Year after Discharge*	*2 Years after Discharge*
30 patients* on Hyphex therapy for 1 year					
Blood pressure S/D mm. Hg	190/121	157/93	161/96	158/94	
Hexamethonium chloride gm./day		2.08	1.77	1.78	
Hydrazinophthalazine gm./day		0.52	0.45	0.49	
10 of these patients with mean control systolic pressures 200 mm. or more†					
Blood pressure S/D mm. Hg	212/130	163/92	162/94	166/96	
Hexamethonium chloride gm./day		2.10	1.80	1.67	
Hydrazinophthalazine gm./day		0.51	0.49	0.46	
10 of these patients on Hyphex therapy for 2 years‡					
Blood pressure S/D mm. Hg	187/117	147/87	150/89	156/91	155/90
Hexamethonium chloride gm./day		1.86	2.09	1.58	1.35
Hydrazinophthalazine gm./day		0.42	0.44	0.36	0.31

The blood pressures recorded in the table were calculated by averaging the mean values for every patient. Every reading in the hospital before therapy was used to obtain the pre-treatment value; the mean number of such readings was 14 per patient. For the other columns the value for each patient is the average of a week's readings starting at the exact interval indicated; this usually amounts to 35 readings. All blood pressures were obtained sitting. The drugs were taken in divided doses, usually 5 times a day. Each value represents the daily intake averaged for the week beginning at the indicated time.

* Patients with surnames beginning with the letters A through M were chosen from analysis. Only those developing delayed toxic reactions to substituted hydrazine and those without continuous daily records for at least one year were excluded. Eight were in malignant stages.

† Four were in malignant stages.

‡ Three were in malignant stages.

iron or copper[14]. A diminution in blood pyruvate or total carbonyl groups was not found[14]. The fraction of renally excreted hydrazinophthalazine which was apparently "bound" to mercaptan decreased as therapy progressed, as though the body stores of some sulfhydryl compound were depleted.

Ingested dihydrazine may have been converted to monohydrazine in the body. When dihydrazine was substituted for monohydrazine, no dihydrazine appeared in the urine but there was a late and lessened excretion of monohydrazine-like material. In addition the therapeutic response was delayed and diminished. Occasionally immediate allergic reactions to the dihydrazine in patients sensitive to the monohydrazine were postponed.

We have been unable to control severe hypertension adequately when either agent was used alone. Hydrazinophthalazine inhibited the tolerance to hexamethonium which has been very commonly observed[21]. The locus of action of hexamethonium ion is well known[11]. The available evidence suggests a different locus and mode of action for hydrazinophthalazine[14]. If so, 2 pathogenetic processes may be concerned in severe hypertension, both of which must be simultaneously affected in order to achieve adequate control.

Summary and Conclusions. Levels of hexamethonium ion were measured in blood and urine of hypertensive patients taking this drug orally and parenterally. About 6% of a single oral dose was excreted in the urine, half within 6 hours. On continuous oral doses the mean renal recovery was 3.5%, whereas on continuous parenteral doses about 10 times as much was excreted. Therapeutic concentrations in plasma averaged 0.37 mg. per 100 cc., a level as high as 16.0 mg. per 100 cc. was found in a constipated azotemic patient who recovered.

Levels of 1-hydrazinophthalazine were likewise measured. The mean recovery of free drug in the urine was 2.9% of a single oral dose; half appeared within 5 hours. On continuous oral doses the mean excretion was 1.1%. Approximately the same amount appeared when the drug was given parenterally. Therapeutic plasma levels averaged 0.023 mg. per 100 cc. At the beginning of treatment about half as much drug was excreted in the urine free as was "bound" to mercaptan; with therapeutic responses the relative amount of free drug increased to more than half of the total. The dihydrazine derivative was not recovered in the urine as such. In 2 years no tolerance to the hypotensive effect of Hyphex has been observed.

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THE GASTROESOPHAGEAL VESTIBULE, ITS NORMAL FUNCTION AND ITS ROLE IN CARDIOSPASM AND GASTROESOPHAGEAL REFLUX*

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The abnormal mechanisms underlying such disorders as cardiospasm and peptic esophagitis are poorly understood, partly because so little is known concerning the normal structure and function of the distal esophagus and the gastroesophageal junction. Recent attempts, for example, to define this junction solely on the basis of mucosal characteristics have been very informative but have also compounded confusion by giving rise to the argument whether a tubular organ, grossly esophagus but lined with gastric mucosa, should be called esophagus or stomach[1,2,18]. We have attempted to define the distal esophagus in terms of its motor function as studied by manometric and radiologic methods in normal human subjects and patients with esophageal disorders.

Direct measurement of esophageal, intraluminal pressures by means of open-tipped catheters placed under fluoroscopic control suggests that the esophagus can be divided into three parts on the basis of its motor response to swallowing[22]. These three parts correspond approximately to anatomic areas defined by Lerche[15]: 1) the body of the esophagus, extending from the upper esophageal orifice to the inferior esophageal sphincter, an inconstant physiologic constriction located about 3 cm. above the gastroesophageal junction; 2) the ampulla,† the terminal few centimeters of the body of the esophagus immediately above the inferior esophageal sphincter; and 3) the gastroesophageal vestibule, the most distal segment, about 2 to 3 cm. in length, lying between the inferior esophageal sphincter and the gastroesophageal junction (Fig. 1.)

In the body of the esophagus, the pressure complex induced by swallowing is distinguished by two features which do not characterize vestibular motility[13,22]. Immediately upon swallowing, a small but abrupt increase in pressure takes place instantly throughout the body of the esophagus[4]. This

*Presented at the meeting of the Association of American Physicians, May 4-5, 1954, Atlantic City, New Jersey.

†The word ampulla as defined by Lerche[15] and as used by many radiologists unfortunately does not have the same meaning. The radiologist's ampulla is neither sharply nor consistently defined; in general, it refers to a distensible portion of the distal esophagus that evacuates contrast media rather slowly. If used in this sense, the radiologist's ampulla probably includes both the ampulla and vestibule of Lerche.

increase in pressure presumably develops when the oropharynx squirts material into the esophagus. Since the pressure change does not appear in the vestibule, it may be inferred that the vestibule is normally closed at the onset of swallowing. The second distinguishing feature of the swallowing complex in the body of the esophagus is the so-called peristaltic pressure

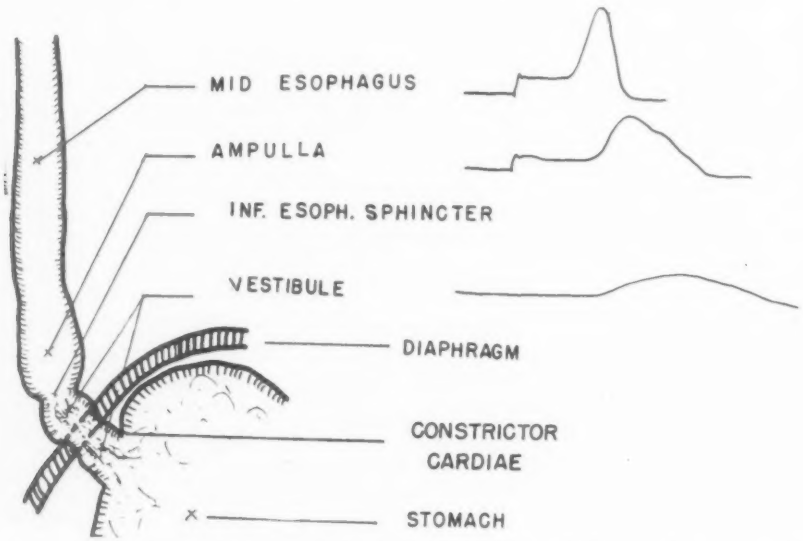

Fig. 1.—Diagram of the structure of the lower two-thirds of the esophagus according to Lerche[15]. The schematic drawings to the right represent the swallowing complexes recorded in the lower esophagus, ampulla and vestibule. (Sanchez, Kramer and Ingelfinger, courtesy of Gastroenterology[22].)

wave. This wave travels down the esophagus at a rate of 2.0 to 2.5 cm. per second, attains maximum pressures ranging between 20 and 100 mm. of Hg, and slows down in the ampullar area. It is not propagated into the vestibule.

The pressure recorded at any point in a hollow viscus depends on several factors: 1) the motility of the viscus at the point of study, 2) the amount and consistency of the visceral content, 3) the rate of inflow as determined by proximal visceral motility, 4) the resistance to outflow as determined by distal visceral motility, and 5) pressure changes within the body cavity occupied by the viscus. The question arises, therefore, to what extent differences in the swallowing complexes in the body of the esophagus and the vestibule are determined, on one hand, by intrinsic differences in motility and, on the other, by resistance to outflow of esophageal content. Indirect evidence bearing on this question is obtained by fluoroscopic observations of a barium swallow performed simultaneously with measurement of intraluminal pressure recorded from two points in the esophagus. Such studies indicate that the peristaltic pressure wave 1) develops and reaches its maximum value *after* swallowed liquid passes the recording tip, 2) is so sharply defined in terms of esophageal area involved that tips no more than 2 cm. apart yield a sequential record of the moving wave.

and 3) slows down just before it reaches the vestibular area[22]. From these observations, it may be inferred that the peristaltic pressure wave is caused by a sharply defined segmental contraction sweeping down the esophagus to form a pressure-tight compartment which pushes the esophageal content ahead of it. If such a contraction with its sharply defined front actually traveled over the vestibule right down to the gastric junction, it should cause pressure changes qualitatively similar to those produced in the body. Actually, vestibular pressures either do not change in response to swallowing or undergo a gradual rise and fall very different in pattern from the swallowing complex in the rest of the esophagus[22]. Radiologic studies, in addition, support the impression that esophageal peristalsis ends several centimeters above the gastroesophageal junction. Since it is unlikely, therefore, that the differences in the swallowing complexes in the body of the esophagus and the vestibule are merely the result of differences in resistance to outflow, it ap-

Fig. 2.—Spot film of the distal esophagus in a patient with moderate cardiospasm. The typical location and segmental character of the narrowed area are illustrated.

pears that the vestibule exhibits characteristic motor function differentiating it from the rest of the esophagus.

Further evidence that the vestibule possesses characteristic and independent motility may be found by examining the function of the distal esophagus in several esophageal disorders, particularly in cardiospasm. Examination of patients with cardiospasm by Roentgen-ray and balloon-kymograph methods show that the motor mechanisms of the smooth muscle of the entire esophagus are deranged[9-11,24]. The distal 2 to 4 cm. of the esophagus appears narrowed and contracted, whereas the body is hypotonic and displays disorganized motility. Radiologically the narrowed area appears exactly in the position of the vestibule (Fig. 2). We have, therefore, advanced the hypothesis that the narrowed area in cardiospasm is indeed an expression of vestibular motor function[22]. This hypothesis may be tested in two ways: 1) can it be reconciled with other observations pertaining to esophageal motility in cardiospasm, and 2) can it be supported by experimental evidence?

The concept that the distal narrow area in cardiospasm is a manifestation of vestibular function not only can be reconciled with the recognized motor phenomena of cardiospasm but would seem to make their complex pattern more understandable. It explains, in the first place, why the narrowed area always appears in the form of a segment, never a thin annular contraction (Fig. 2). Second, the idea of Meltzer[16] and Hurst[8] that the narrowed segment is caused by failure of relaxation rather than spasm makes physiologic sense if it is accepted that the vestibule normally changes from a closed to an open state as an integral part of the swallowing act, but which, in cardiospasm, does not exhibit this normal response. Third, evidence is now unequivocal[4,10,11,23] that the motor disorder in cardiospasm is not confined to the distal narrowed segment but that the body of the esophagus, even in early cases, is affected by decreased tone and impaired propulsive motility. Assuming that cardiospasm is caused by a single disorder, it would be hard to explain how such a disorder could cause a relatively hypotonic state in the upper esophagus and a relatively hypertonic condition in the distal portion unless it is accepted that these two esophageal regions normally may exhibit different motor functions.

Pathologic studies have shown damaged and diminished numbers of myenteric plexus cells in the esophageal wall in cardiospasm, a finding suggesting that the underlying disorder in this disease is a defect in the cholinergic innervation of the esophagus[6,14,19]. In previous studies[11,12] we have described an abnormally sensitive response of the body of the cardiospastic esophagus to cholinergic stimulation with drugs. After as little as 1.5 to 6.0 mg. of Mecholyl intramuscularly, the distal half of the body of the esophagus undergoes a tetanic and frequently violent contraction. This hypersensitive response to a parasympathomimetic agent has been interpreted on the basis of Cannon's law[5] as providing further evidence of absent or impaired postganglionic cholinergic innervation of the cardiospastic esophagus[7,11,12,23].

The response of the vestibule to Mecholyl was not studied in our former investigations, but Roark[20], of the University of Virginia, suggested that if the vestibule and the body of the esophagus normally function differently, the response of these two areas to cholinergic stimulation should also differ. Radiologic observations of the vestibule have been made in 3 patients with cardiospasm given Mecholyl. In one case with advanced sigmoid dilatation of the esophagus, no obvious

change occurred. In the second, a moderately advanced case, Mecholyl definitely appeared to widen the diameter of the vestibule. In the third, an early case of a patient who suffered considerable esophageal pain, retching and belching after Mecholyl, such a wide dilatation of the gastroesophageal junction took place as to suggest either marked relaxation of the vestibule, as bule, and that, indeed, an opposite effect may develop.

The apparently different effect of Mecholyl on the vestibule and the body of the esophagus in cardiospasm implies that the vestibule possesses pharmacologic as well as physiologic and structural identity. To explore this possibility further, the effect of strong anticholinergic stimulation on the normal

A B

Fig. 3.—A. Spot film of distal esophagus in early case of cardiospasm. B. Spot film of same case 3 minutes after 6 mg. of Mecholyl intramuscularly. At the time picture was taken, the patient was belching and retching.

is seen when a normal person belches[25], or a sudden diaphragmatic herniation, a most unlikely result in a patient with cardiospasm (Fig. 3). The tentative impression, based on these radiologic and fluoroscopic studies, is that the spasmogenic effect of Mecholyl in cardiospasm does not extend to the vesti- esophagus of 2 male subjects, aged 63 and 71, was studied. After 35 mg. of intravenous Banthine was given to these subjects,* the swallowing complexes recorded from the body of the esophagus were dramatically altered (Fig. 4). The initial intraluminal pressure rise that occurs immediately on

*The effect of Banthine in these doses is believed to be anticholinergic, not ganglion blocking. The subjects had no signs of vascular collapse when they were tilted upright.

swallowing was, as might be expected, unaffected. All vestiges of the peristaltic pressure wave were, however, eradicated. The effect of anticholinergic stimulation on the vestibular pressure record was not striking, but this finding must be interpreted in the light of supplementary information provided by radiologic observations. Before Banthine was given, esophageal function was studied by giving barium swallows to the subjects lying in a supine position. As is normally the case, esophageal peristalsis swept the esophagus clear of barium in each instance within less than 30 seconds after barium suspension was swallowed. After intravenous Banthine, however, swallowed barium was retained in the esophagus for a period of more than 10 minutes, the esophageal lumen appeared widened, and purposeless and disorganized peristaltic contractions reminiscent of those seen in cardiospasm were seen to indent the esophageal lumen. When the subjects were tilted upright, barium was still retained in the esophagus, a fluid level was demonstrated, and the only cause that could be discovered for the retention of barium suspension in the face of gravity was a nonrelaxing vestibular lumen (Fig. 5).

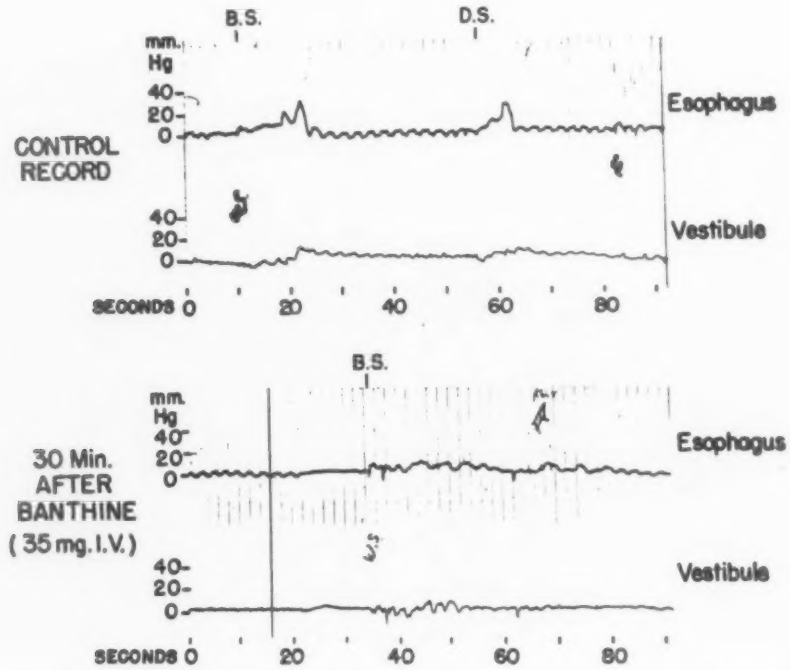

Fig. 4.—Records of intraesophageal pressure obtained in the body and vestibule of a normal esophagus. Following BS (barium swallow) in the control record, essentially normal pressure complexes are seen. Following BS in the post-Banthine record, the initial pressure rise that occurs immediately on swallowing is seen, but the peristaltic pressure wave is absent.

The effects of Mecholyl in cardiospasm and those of Banthine in normal subjects suggest that the vestibule and the body of the esophagus react inversely to cholinergic stimuli. These

Fig. 5.—Effect of strong anticholinergic stimulation on the motility of a normal esophagus. A. Control picture showing empty esophagus 30 sec. after barium swallow. Subject supine. B. Effect of Banthine (30 mg., intravenously) in supine subject. A barium swallow is retained in esophagus for 10 minutes; esophageal peristalsis is uncoordinated and not propulsive. C. Effect of Banthine in upright subject. A barium swallow is retained in the esophagus apparently because of nonrelaxation of vestibular area.

Fig. 6.—Chalasia of vestibule. When this patient was tilted from the erect to the supine position, a barium suspension previously confined to the stomach rapidly regurgitated into the esophagus as shown in spot films A and B taken in rapid succession.

Free gastroesophageal reflux had been recognized in this patient for some time but no evidence of hiatus hernia had been found. Nevertheless, at the time of a hemigastrectomy and vagectomy undertaken for duodenal ulcer, the surgeon made special efforts to "snug up" the hiatus. The postoperative films shown here suggest that the condition of the hiatus had little to do with this patient's pre- and postoperative gastroesophageal reflux.

inverse reactions of the esophagus and the vestibule provide evidence, furthermore, that propulsive and sphincteric mechanisms in the human gut are subject to reciprocal cholinergic stimuli, a relationship shown in animal experiments but difficult to establish in man[26].

In cardiospasm, the vestibule exhibits achalasia, that is, failure of relaxation. The opposite condition, namely, chalasia or failure of the vestibule to remain contracted between swallows, also exists[3,21] and may have wider implications than achalasia. Chalasia has been recognized as a disorder occurring in infants and responsible for abnormal gastroesophageal regurgitation[17]. As shown in Fig. 6, chalasia may be striking in adults who have no evidence of hiatus hernia.

The various mechanisms underlying gastroesophageal reflux are obscure. At present a major role in the prevention of gastroesophageal reflux is credited to the diaphragm and to angulation of the lower esophagus; intrinsic esophageal mechanisms are either not considered or assigned secondary roles. The exact mechanisms of diaphragmatic control of gastroesophageal reflux have, however, not been elucidated, and the interrelationship of vestibular function and diaphragmatic herniation is as yet an unstudied field. The existence of gastroesophageal reflux in patients who have absolutely no evidence of diaphragmatic herniation but who appear to suffer from chalasia suggests that vestibular dysfunction underlies some types of gastroesophageal reflux. Even in patients with clear-cut diaphragmatic hernia, the adequacy of the vestibular mechanism may prove as important as the direct action of the diaphragm in determining whether or not material regurgitates from the stomach into the esophagus.

Summary. 1. Radiologic and manometric studies indicate that the distal 2 to 3 cm. of the normal human esophagus, the vestibule, has characteristic motor function.

2. The effect of cholinergic and anticholinergic stimulation on esophageal motility suggests that the body of the esophagus and the vestibule react inversely to such stimuli.

3. The concept of a vestibule permits explanation of the several motor abnormalities of cardiospasm in terms of a single neurogenic disorder.

4. The vestibule may also play an important role in the control of gastroesophageal reflux.

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THE EFFECT OF PROTEOLYTIC ENZYMES ON THE PHYSICAL AND CHEMICAL CHARACTERISTICS OF THE TRACHEOBRONCHIAL SECRETIONS OF PATIENTS WITH POLIOMYELITIS

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Atelectasis is a frequent and serious complication in patients with poliomyelitis who have been subjected to tracheotomy and are in mechanical respirators. Although many factors contribute to the development of the atelectasis, the accumulation of viscous secretions in the respiratory tract of the patients is among the most important. Therefore, any agent which will reduce the viscosity of these secretions may facilitate their removal and be useful in the prevention or treatment of atelectasis. Proteolytic enzymes have been advocated as a means of increasing the fluidity and the ease of removal of the secretions of the respiratory tract[1,4,6,8,9]. In this paper we are reporting observations on the physical and chemical properties of tracheobronchial aspirate before and after administration of trypsin and streptokinase and streptodornase.

Methods and Materials. The subjects were hospitalized in the Municipal Contagious Disease Hospital of Chicago. Each of them had acute poliomyelitis with weakness of the pharyngeal and respiratory muscles, had been subjected to tracheotomy and had been placed in a mechanical respirator. The enzymes that were used were crystalline trypsin* derived from mammalian pancreas, and streptokinase and streptodornase† derived from a strain of *Streptococcus pyogenes*.

Secretions were obtained from the trachea following the administration of the enzymes in an aerosol and from the same person following the nebulization of isotonic sodium chloride without the enzymes.

Solutions were placed in a plastic vaponefrin nebulizer and were aerosolized by a stream of oxygen flowing at a rate of 6 liters per minute. The outflow tract delivered the aerosol directly over the tracheotomy orifice. Generally the treatment was completed by 30 minutes, but occasionally it took one hour. After administration of the vapor for 15 minutes, collection of the tracheal secretions was begun and continued for 2 hours by tracheobronchial aspiration at 15-minute intervals. If the atomization was not finished when the collection period was begun the administration was continued between aspirations until it had been completed. During each 2-hour period of collection, 25 cc. of 0.85% sodium chloride solution was instilled into the trachea,

*Kindly supplied by Armour Laboratories, Chicago, Illinois. Trade name "Tryptar."

†Kindly supplied by the Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York. Trade name "Varidase."

since aspiration of the tracheal secretions had been found to be unsuccessful and traumatic when no supplemental solution was used. The quantity used during each control and experimental period was the same. The rate of saline instillation during the 2-hour period was approximately the same rate of administration as used routinely in the tracheotomy care of these patients. After the first 2-hour collection had been completed approximately 30 minutes were allowed to elapse before the second solution was placed in a nebulizer. After 15 minutes of administration of the second aerosol, the second 2-hour period of collection of tracheal secretions was begun. The aerosol solutions used were: (1) 250,000 units (250 mg.) of trypsin in 6 cc. of Sorensen's phosphate buffer solution (pH 7.1); (2) 100,000 units of streptokinase and 25,000 units of streptodornase in 6 cc. of 0.85% sodium chloride solution, and (3) 6 cc. of 0.85% sodium chloride solution.

In the experiments performed to determine the effects of trypsin, 8 patients received saline aerosol in both the first and second collection period. In 8, the saline aerosol was administered first and the trypsin second, and in 9 patients, the aerosols were administered in the reverse order. In the second group of experiments in which the effects of the streptococcal enzymes were measured, the enzymes were administered to 7 patients in the initial period, and in 9 the saline was given first. The patients in the second group of experiments were more chronically ill than those in the first group of experiments, and, therefore, no comparison of results in these 2 groups was made.

The samples obtained were studied *in vitro* to determine the effect of the enzymes on viscosity, volume, per cent sediment, pH, and total nitrogen. Gram and Wright stains, as well as other bacteriologic studies were frequently done on the patients' secretions prior to the collections.

For the measurement of the viscosity, an Ostwald viscosity pipette (No. 70710) was placed in a constant-temperature water bath at 26° C. and the time of outflow was measured. Three successive readings that agreed within 5 seconds of each other were obtained before the measurements were accepted. The density of the material was determined by weighing, and the viscosity was calculated by means of the following formula: $\frac{Vs}{Vw} = \frac{DsTs}{DwTw}$ where V represents viscosity, D density, T time of outflow, s tracheal secretions, and w water. Freezing and thawing the secretions to disrupt cellular material made no appreciable difference in the viscosity of a number of samples which were studied in this manner.

The total volume of secretions collected during each period was measured, and an aliquot was centrifuged for 10 minutes at 1500 RPM, following which the per cent sediment was observed. The pH was determined by the Beckman pH meter and the use of nitrazine paper. The total nitrogen content of each specimen was determined by the micro-Keldahl method. The nitrogen content of instillate was pre-determined. Some of the samples were tested on a gelatin film for residual tryptic activity.

Results. In Table 1, the volume and the per cent of sediment in the secretions collected after the use of aerosol trypsin or aerosol sodium chloride solution are presented. The differences in volume and per cent sediment of the various groups were not statistically significant, as shown by calculation of the "t" values.* The mean total nitrogen value of a sample when trypsin was used was 10 mg. higher than the mean total nitrogen of a sample when sodium chloride was used. The increase is accounted for by the amount of nitrogen in 250,000 units of trypsin which was found to be 12.2 mg.

The pH of the tracheal secretions varied between 5 and 8.4, the average being 7.1. The pH of the secretions in the groups of patients receiving either saline or one of the proteolytic enzymes was similar.

Table 2 indicates an apparent decrease in viscosity when trypsin was used. Since the volume, per cent sediment and total nitrogen were constant, all of the sodium chloride groups were combined and compared with the combined trypsin group (Table 2). The difference in viscosities between the saline and trypsin groups was significant (Chi square = 5.2, P = 0.02). The difference in viscosity between all

*In this study a P value of 0.05 or less was considered to be significant.

of the specimens collected during the first period and those collected during the second period was not significant. There was no significant difference in viscosity between any 2 saline groups or among any combination of saline groups. The action of aerosol trypsin as administered in these patients was apparent for only 2 hours, as indicated by the lack of effect upon the secretions collected during the second period when saline followed the administration of trypsin.

Table 3 indicates that there was also a decreased viscosity during the administration of the streptococcal enzymes as compared to isotonic sodium chloride ($P = 0.01$). In addition, it can be seen from Table 3 that there was a significant decrease in per cent of sediment and mean total nitrogen per cc. when aerosol streptococcal enzymes were used in the second period. The effect produced by the streptococcal enzymes lasted for at least 4 to 5 hours as shown by the similarity of the specimens in the second saline group as compared to the first streptococcal enzyme group.

Discussion. It has been previously established that the effect of trypsin on the tracheobronchial secretions is, in part, due to mucolysis[3]. Streptokinase-activated enzymes, however, are less effective in mucolysis but are more potent in fibrinolysis[7]. Streptodornase acts directly upon a substrate of desoxyribonucleoprotein and desoxyribonucleic acid[5]. In addition, the streptococcal enzymes incite a local outpouring of fluid and phagocytes at the site of application[2]. This study was undertaken to see whether proteolytic enzymes which are active *in vitro* have a similar effect *in vivo*.

The results have demonstrated that

TABLE 1.—THE EFFECT OF TRYPSIN ON THE VOLUME AND PER CENT OF SEDIMENT OF TRACHEOBRONCHIAL SECRETIONS

Treatment			*Volume (cc.)*			*Per Cent Sediment*		
Period	*Solution*	*Number of Observations*	*Mean*	*Range*	*Standard Deviation*	*Mean*	*Range*	*Standard Deviation*
First	Sodium Chloride	8	22.5	14–39	±11.0	20.2	6.8–47.4	±17
Second	Sodium Chloride	8	17	8–30	±18.6	30.8	7.2–62.5	±18
First	Sodium Chloride	8	27	5–60	±18.6	30.6	3.6–87.0	±23.5
Second	Trypsin	8	23	5–38	±12.4	32.5	3.5–92.1	±32.6
First	Trypsin	9	26	5–47	±19.2	17.0	8.0–27.7	±7.1
Second	Sodium Chloride	9	27	5–48	±11.7	17.5	9.0–28.6	±7.4

TABLE 2.—THE EFFECT OF TRYPSIN ON THE VISCOSITY OF TRACHEOBRONCHIAL SECRETIONS

Treatment and Period	*Number of Specimens*	*Number with Viscosity of* 1.0–1.5	>1.5
First Treatment Period			
a) Sodium Chloride	8	4	4
b) Sodium Chloride	8	3	5
c) Trypsin	9	7	2
Second Treatment Period			
a) Sodium Chloride	8	2	6
b) Trypsin	8	6	2
c) Sodium Chloride	9	3	6
Total Sodium Chloride	33	12	21*
Total Trypsin	17	13	4*

* $P = 0.02$

TABLE 3.—THE EFFECT OF THE STREPTOCOCCAL ENZYMES ON THE VOLUME, SEDIMENT AND NITROGEN CONTENT OF TRACHEOBRONCHIAL SECRETIONS

Treatment	*Number of Observations*	*Number with Viscosity of*		*Volume*			*Per Cent Sediment*			*Total Nitrogen per cc. of Specimen*		
		1 to 1.5	*>1.5*	*Mean*	*Range*	*Standard Deviation*	*Mean*	*Range*	*Standard Deviation*	*Mean*	*Range*	*Standard Deviation*
Sodium Chloride First Period	9	0	9	30*	18–69	±12.6	27.6*	7–75	±15.7	27.7*	5–49	±12.1
Streptokinase-Streptodornase Second Period	9	6	3	46*	25–105	±24	12.1*	1.5–22	±7.2	15.4*	2.3–27	±7.9
Streptokinase-Streptodornase First Period	7	6	1	31†	13–75	±20	4.7†	1.3–14	±4.1	13.8†	4.5–26	±9
Sodium Chloride Second Period	7	4	3	31†	17–55	±12.4	4.6†	1.4–12	±4.2	12.8†	4.0–21	±6.2

Volume expressed in cc.
Nitrogen values expressed in mg.
* P = 0.05
† P = no significant difference

aerosolized trypsin administered to patients through a tracheotomy tube decreased the viscosity of tracheal secretions without changing the volume, per cent of sediment or nitrogen content per cc. Thus the main action was that of alteration of the physical state of the organic material. Since the aspirated secretions when tested failed to show residual tryptic activity it is likely that the digestion occurred *in vivo*.

Because there was a significant difference in the amount of sediment, total aspirate volume and nitrogen concentration in the secretions which were collected after the streptococcal enzymes were administered, whereas the volume, per cent of sediment and nitrogen in the fluid collected after administration of trypsin or sodium chloride were constant, it is probable that the principal effect of the former enzymes was to increase bronchial secretions. It may be noted that the viscosities in the sodium chloride control groups in the trypsin experiment were less than those in the sodium chloride group in the streptococcal enzyme experiment, probably because the patients in the latter experiment had been tracheotomized for a considerably longer time and had recovered from the stage of copious secretory activity frequently seen for several weeks following tracheotomy. It may have been easier, therefore, to demonstrate a bronchorrheic effect with the streptococcal enzymes than it was for trypsin.

The effect of streptococcal enzymes persisted at least 4 hours and probably longer in a number of patients since the secretions in the control period following the use of streptococcal enzymes were almost identical with those collected during the period of enzyme administration. On the other hand, the trypsin effect was not as persistent.

Since the tracheal aspirates were mainly composed of mucin, cellular debris, bacteria and occasionally fibrin, it is likely that the major activity of trypsin was mucolysis. On the other hand, if the cellular debris was modified by the streptodornase, or if there was any fibrinolysis accomplished by activation of plasminogen by streptokinase, the effect was not demonstrable in the face of the bronchorrhea which occurred. In spite of the apparent difference in mode of action of the 2 types of enzymes, the resulting reduction of viscosity could conceivably be of benefit to some patients with inspissated secretions in the bronchial tree. It is also possible that some patients, particularly those with decreased tussive force or excessive secretions prior to treatment, may be worsened rather than benefited by the bronchorrhea induced by the streptococcal enzymes. Even the decreased viscosity following trypsin may be harmful in similar patients by facilitating the spread of material in the distal bronchi if adequate drainage cannot be established. The determination of the worth of these compounds in the treatment of patients will depend upon carefully controlled studies on patients in need of tracheobronchial aspiration.

Summary and Conclusions. 1. The effect of the proteolytic enzymes trypsin and streptokinase-streptodornase on some physical and chemical characteristics of tracheobronchial secretions of patients with acute poliomyelitis with weakness of the pharyngeal and respiratory muscles who had been subjected to a tracheotomy and placed in a mechanical respirator, is reported.

2. Isotonic sodium chloride solution was used as a control. During the course of experiments isotonic sodium chloride was administered 49 times, trypsin 17 times, and the streptococcal

enzymes 16 times. The following measurements were made: viscosity, volume, per cent of sediment, pH and total nitrogen.

3. The principal action of trypsin on the secretions was that of digestion which was apparent for only 2 hours under the conditions of the experiments. The principal effect of the streptococcal enzymes was the production of bronchorrhea, which was apparent for at least 4 hours. A digestive action of the streptococcal enzymes was not demonstrated.

ACKNOWLEDGMENTS: We wish to thank the staff of the Municipal Contagious Disease Hospital of Chicago for their cooperation, and Mr. Joseph Seto for technical assistance.

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THE USE OF TRYPSIN OR STREPTOKINASE-STREPTODORNASE FOR THE THERAPY AND PREVENTION OF ATELECTASIS

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In a preceding paper[2] it was shown that trypsin* administered by the aerosol route decreased the viscosity of the tracheobronchial secretions of patients who had acute poliomyelitis and on whom tracheotomy had been performed. The main action of the trypsin was digestive since it decreased the viscosity of these secretions without changing the volume, per cent of sediment or total nitrogen. On the other hand, the streptococcal enzymes,† streptokinase and streptodornase, decreased the viscosity but at the same time increased the volume of secretions and decreased the per cent of sediment and total nitrogen per cc. Therefore the principal action of the streptococcal enzymes was interpreted to be the production of a bronchorrhea. Because of the frequency and severity of atelectasis in patients who have had a tracheotomy and have been receiving artificial respirations[3] proteolytic enzymes have been advocated as a means of reducing the viscosity of the secretions of the respiratory tract and to facilitate their removal[5-8]. Since the course of the disease is variable and unpredictable it was felt that a controlled study of the clinical usefulness should be done in which the effects of trypsin, the streptococcal enzymes and a nonproteolytic solution were compared.

Methods and Materials. The patients reported in this study were hospitalized in the Municipal Contagious Disease Hospital of Chicago. Each of the patients had acute poliomyelitis with weakness of pharyngeal and respiratory muscles and therefore had been subjected to a tracheotomy and placed in a mechanical respirator. Since the patients to whom the enzymes were administered were critically ill, a rigid control of total therapy was impossible. However, the following studies were made: 1) an attempt to evaluate the effectiveness of aerosol administration of the enzymes in the prevention of respiratory tract complications, 2) a controlled study involving the use of a placebo to evaluate the usefulness of intratracheal or intrabronchial instillation of trypsin in the treatment of atelectasis.

In the first aspect of the study, one of four aerosol treatment regimens was given prophy-

*Supplied by Armour Laboratories, Chicago, Illinois. Trade name "Tryptar."

†Supplied by The Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York. Trade name "Varidase."

lactically to each of 24 patients chosen by strict alternation at the time of tracheotomy. Thus there were 4 groups of 6 patients each in the series. Patients in the first group, who served as nonenzyme controls were given aerosols of 2.5 mg. per kg. of polymyxin B sulfate* in 0.85% normal sodium chloride during a 24-hour period. The second group received 250,000 units to 1,000,000 units of trypsin in phosphate buffer each 24 hours. In the third group, a similar dose of trypsin was combined with the same dose of polymyxin as used in the control group. The fourth group received 2.5 mg. per kilogram of polymyxin plus 100,000 to 400,000 units of streptokinase and 25,000 to 100,000 units of streptodornase over a 24-hour period. The patients were started on polymyxin in an attempt to limit and make consistent the antibiotic treatment in the face of a high incidence of infections of the tracheobronchial tree with *Pseudomonas aeruginosa* among similar patients who were in the hospital at the start of the study. Other antibiotics were withheld for at least 48 hours, and were given parenterally or by stomach tube when they were required. The group of patients who received trypsin without polymyxin was included in order to distinguish the enzymatic from the antibiotic effect. The original regimens were adhered to for one to 9 days, but were subsequently altered either by addition of other antibiotics or terminated by the patient's improvement or death.

The aerosol solutions, which were placed in a plastic vaponefrin nebulizer, were aerosolized by a stream of oxygen flowing at a rate of 6 liters per minute and delivered by an outflow tube directly over the tracheotomy orifice. Generally the treatment was complete by 30 minutes, but occasionally it took as long as one hour.

In the second phase of the study an evaluation of the effectiveness of direct instillation of trypsin into the bronchi was sought. Initially 250,000 units of trypsin in 4 cc. of phosphate buffer was instilled through a rubber tracheal catheter into either the right or left main bronchus. After 3 patients had received a total of 8 treatments, it was decided to compare results obtained from instillation of the enzymes with the instillation of 4 cc. of isotonic sodium chloride in alternate patients. The alternation was accomplished by having the laboratory staff supply the solution so that the clinicians were unaware of whether the enzyme or sodium chloride was being used. In order to minimize variation, one physician gave 27 of the 31 instillations. Because several serious adverse reactions were observed with the aerosol streptococcal enzymes, it was decided not to instill them directly into the bronchi.

Before administration of either enzyme or control solution, adults were given 50 mg. and children 25 mg. of diphenhydramine hydrochloride (Benadryl) or tripelenamine hydrochloride (Pyribenzamine), either orally or intramuscularly. In addition, 0.6 gm. of acetylsalicylic acid was often given. In some patients the dose of the antihistaminic was repeated one and 2 hours after the administration of the enzymes. The instillate was directed into the upper or lower lobes by raising or lowering the foot of the respirator and into the right or left bronchi by turning the patient on the appropriate side for 15 minutes. Tracheobronchial aspiration was delayed for 20 minutes unless untoward reactions required earlier aspiration. At least 48 hours were allowed to elapse before the procedure was repeated. The necessity for a mechanical respirator frequently made it difficult to detect the site of atelectasis. Accordingly, unless clinical or roentgenographic evidence indicated which lung was involved the first instillation was into the right main bronchus and subsequent instillations were given alternately into the left and right sides.

Results. AEROSOL TREATMENT. The criteria used to evaluate the effectiveness of the prophylactic aerosol regimen were: duration of fever, number and severity of cyanotic episodes, clinical roentgenographic evidence of atelectasis, or both, ability to breathe without the respirator, ease of aspiration of secretions and the number of deaths in each group.

In some patients, aerosol trypsin with or without polymyxin, appeared to facilitate the aspiration of the secretions. Aside from this, as seen in Table 1, there was no apparent difference in the clinical course of the patients receiving trypsin with or without polymyxin as compared to those receiving polymyxin in sodium chloride. Similarly, no favorable effect was demonstrated from the streptococcal enzymes.

*Kindly supplied by Burroughs Wellcome & Co., Inc., Tuckahoe, New York. Trade name "Aerosporin."

Two deaths occurred in each of the 2 groups of patients given trypsin and 3 deaths each among the patients given no enzyme or streptococcal enzymes.

No adverse reactions were observed in patients who received aerosol trypsin or saline. On the other hand, 3 patients who received the aerosol streptococcal enzymes experienced a moderate to severe reaction (Fig. 1). These were characterized by the following symptoms: 1) fever, 2) dyspnea, 3) cyanosis, 4) bronchorrhea, 5) sensation of choking, and 6) an increased dependence on the respirator. One patient who received these aerosols before the alternation was begun developed pneumonia as a result of this procedure (Fig. 2). Two patients had received the streptococcal enzymes previously without any reaction, but the one patient who developed pneumonia had not.

TABLE 1.—THE EFFECT OF AEROSOL ADMINISTRATION OF PROTEOLYTIC ENZYMES ON THE CLINICAL COURSE OF PATIENTS WITH A TRACHEOTOMY

Group	*Number of Patients*	*Died, Number*	*Lived*	
			*Number in Whom There was no Evidence of Serious Respiratory Complications while Receiving Aerosol**	*Number in Whom There was Persistent Respiratory Distress while Receiving Aerosol**
Control	6	3	1	2
Trypsin without Antibiotics	6	2	2	2
Trypsin with Antibiotics	6	2	2	2
Streptococcal Enzymes with Antibiotics	6	3	2	1

* As determined by criteria outlined in text.

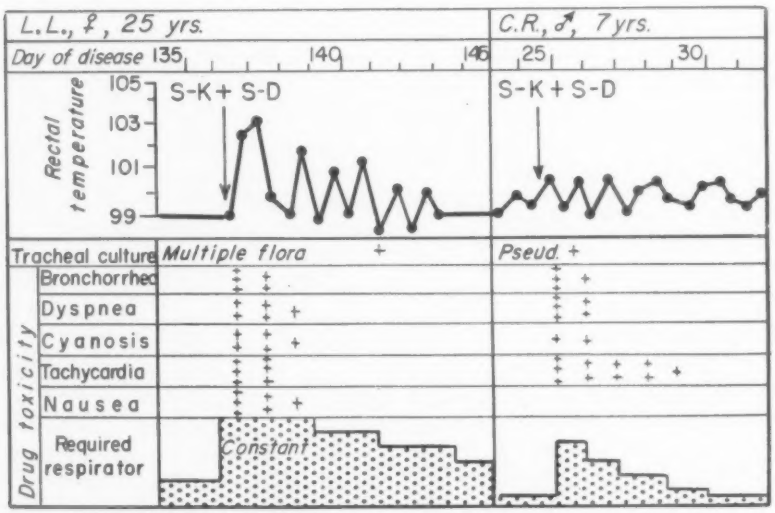

Fig. 1.—Reactions following the aerosol administration of streptococcal enzymes.

INSTILLATION THERAPY. To evaluate the efficacy of direct trypsin instillation, the following criteria were used: 1) a decrease in the temperature to 100° F. (rectal) or below, which occurred

within 48 hours and lasted for at least 24 hours, 2) general improvement in the patient's condition (disappearance of cyanosis, improvement in breathing and ability to remain out of the respirator for a longer period of time), 3) improvement in areas of atelectasis as determined by roentgenograms, 4) thinning of secretions, and 5) the occurrence of untoward reactions.

As seen in Table 2, 11 patients received trypsin for a total of 21 instillations. The temperature was elevated before administration in 17 instances and on 2 occasions the temperature dropped to 100° F. (rectal), or below, for 24 hours or more within 48 hours of the time of instillation. In 2 patients there was definite clearing of pneumonic or atelectatic areas, or both,

TABLE 2.—DIRECT INSTILLATION OF TRYPSIN SOLUTION INTO THE BRONCHIAL TREE

Treatment Group	*Number of Patients*	*Number of Instillations*	*Afebrile within 48 Hours*	*General Improvement*	*Improvement in Roentgenogram*	*Adverse Reactions*
Trypsin, before onset of Controlled Experiment	3	8	1 patient	After 3 Instillations	No roentgenogram taken	After 4 Instillations
Trypsin, after onset of Controlled Experiment	8	13	1 patient	After 6 Instillations	Definite improvement in 2 out of 4 patients who had roentgenograms taken	After 7 Instillations
Sodium Chloride	9	10	0	After 1 Instillation	No improvement in the 2 patients who had roentgenograms taken	0

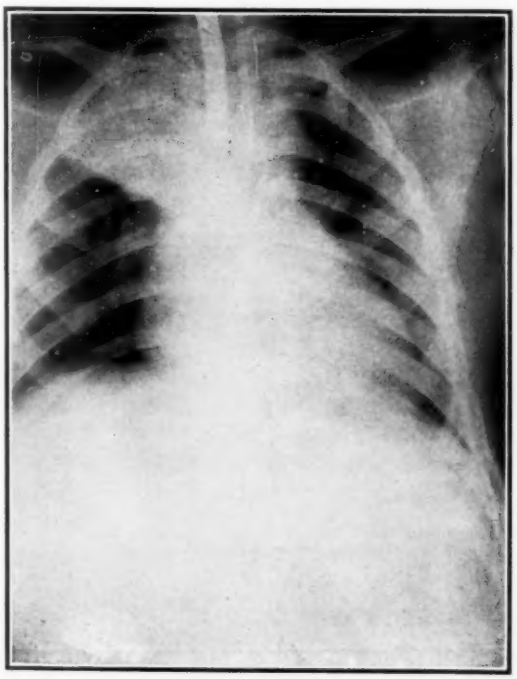

Fig. 2.—Pneumonia and atelectasis complicating poliomyelitis.

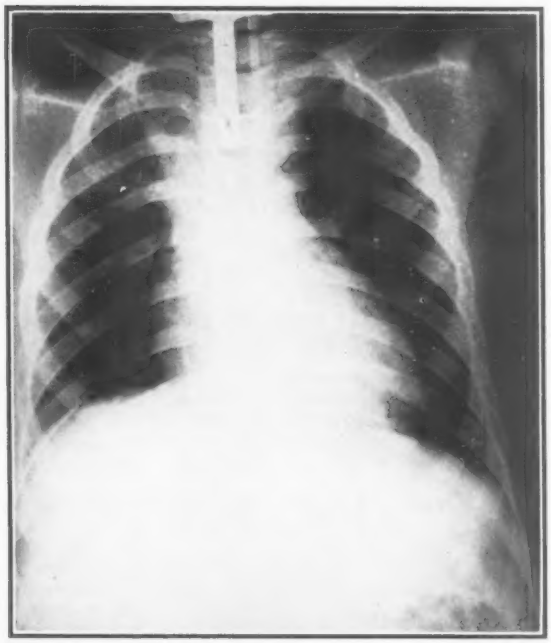

Fig. 3.—Clearing of pneumonia and atelectasis 6 days following repeated intratracheal instillation of trypsin.

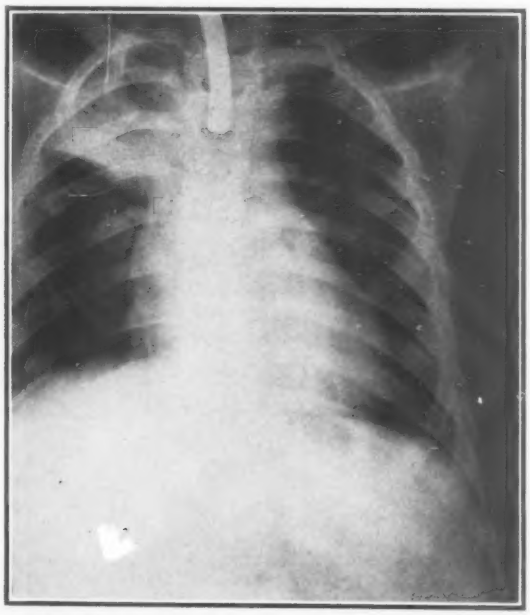

Fig. 4.—Pneumonia and aelectasis in the same patient as in Fig. 2, 2 days following the aerosol administration of the streptococcal enzymes.

within a few days after treatment (Figs. 3, 4). Improvement in the general condition was seen after 9 instillations in 8 patients. Bronchorrhea occurred 11 times, and, as a result, frequent suctioning and almost constant observation were necessary. The increased flow of secretion usually disappeared within a few hours, although it occasionally lasted 48 hours. There was no difference in results in the patients who received trypsin before the controlled experiment as compared to those who received it after the controlled experiment had been started. Nine patients received a total of 10 instillations of isotonic sodium chloride. On 8 occasions fever was present and in no instance was there a change in temperature following therapy. General improvement was observed on only one occasion. In 2 cases roentgenograms were taken and in neither was there any change in the atelectatic areas following therapy. Four of the patients who received sodium chloride and 2 who were given trypsin died.

Eleven of the 21 instillations of trypsin were accompanied by adverse reactions. The symptoms are listed below in the order of frequency: 1) dyspnea (9 times), 2) choking sensation or retromanubrial burning sensation (9 times), 3) cyanosis (twice), 4) fever and cyanosis (once). Most of the reactions occurred within 30 minutes of instillation although the rise in temperature was delayed a few hours (Fig 5). The symptoms were usually mild and transient and did not usually necessitate interruption of therapy. In one case the trachea had to be suctioned 3 minutes after instillation because of dyspnea and cyanosis but in most cases the aspiration could be delayed for 20 minutes. In the few cases in which more severe symptoms were seen, immediate aspiration revealed profuse bronchorrhea and frequent suctioning ameliorated the symptoms. Occasionally additional antihistaminics were given. Adverse reactions did not occur with the 10 control administrations of saline.

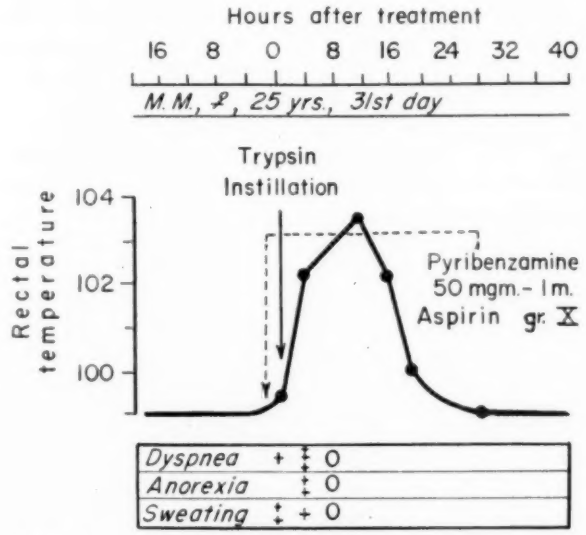

Fig. 5.—Reaction following the intratracheal instillation of trypsin.

Discussion. Previous studies have shown that trypsin has an effect on tracheobronchial secretions *in vitro*[2], and the mechanism of action is believed to be such that it should be beneficial *in vivo*[4]. Previous reports indicate that in certain situations such a benefit may occur[5-8].

In this study it was shown in patients with poliomyelitis who had very poor tussic force and a tracheotomy, and who required a mechanical respirator, that prophylactic aerosol administration of trypsin at intervals was not effective in preventing the occurrence of and death from respiratory tract complications. Since the groups were relatively small it cannot be stated that trypsin was completely without effect although the differences were so small that the practical usefulness of this procedure was obviously slight, even if there was an effect. On the other hand, the use of the streptococcal enzymes seemed to have been without any effect and because of the tendency of these agents to produce bronchorrhea those patients without an adequate cough mechanism may well have been harmed. It is well to remember, however, that if an adequate cough mechanism is present this effect may not be detrimental and may even be beneficial. The occurrence of adverse reactions following aerosolization of the streptococcal enzymes may have been caused by an accentuation of the bronchorrheagenic effect, although they may also have been in part caused by prior sensitization since 2 of the reactions occurred in patients who had tolerated the compounds a week before. Hazlehurst has indicated the sensitizing capacity of these enzymes[1]. That the direct instillation of trypsin into the bronchi may have been more effective than saline was shown by the more frequent improvement and fewer deaths. However, these differences were not statistically significant and at best may indicate a trend that would have to be established on a larger series. However, since in most patients the site of pathology was not accurately known and the instillations were made at random, as far as the right and left bronchi were concerned, the fact that approximately 50% of the instillations were followed by improvement may indicate a degree of success when the material was actually instilled into the proper site.

In spite of the fact that antihistaminics and acetylsalicylic acid were given prophylactically to all patients before the direct instillation of trypsin into a main stem bronchus, a transient adverse reaction was produced in 52.4% of patients. The high incidence of these complications following direct instillation as compared to the absence following the aerosolization of trypsin may have been related to the concentration of the drugs in the former instance. Such untoward reactions may have decreased whatever clinical benefit the compound was exerting. However, because the net effect seemed to be one of some worth the direct instillation of trypsin into the bronchi of the involved segment can be recommended in atelectasis. Every attempt should be made to determine the site of pathology and limit the instillation to the appropriate area.

Summary and Conclusions. 1. Twenty-four patients with acute poliomyelitis, pharyngeal and respiratory weakness, who required a tracheotomy and the aid of a mechanical respirator, were given aerosol treatment after selection by alternation into one of the 4 following groups: 1) polymyxin plus isotonic sodium chloride, 2) trypsin, 3) polymyxin plus trypsin, and 4) polymyxin plus streptokinase-streptodornase. There was no apparent difference in the clinical course of patients

in the first 3 groups. Three patients experienced a severe reaction as a result of aerosol inhalation of the streptococcal enzymes. One of these patients developed a bronchopneumonia shortly after administration of these enzymes. Therefore, streptococcal enzymes are not recommended in this type of patient.

2. Eleven patients received 21 direct instillations of trypsin into the tracheobronchial tree when atelectasis was believed to be present, and 9 patients received 10 direct instillations of isotonic sodium chloride. Direct instillation of trypsin appeared to be beneficial following 42.9% of instillations. There was a similar improvement in the clinical course in 10% who received the direct instillation of saline. Reactions characterized by transient increase in respiratory difficulty occurred after 52.6% of the direct instillations of trypsin, but they were usually mild. There were no similar reactions in the sodium chloride group.

3. Since trypsin appeared to be effective in 42.9% of the instillations in a situation where one-half of the instillations were likely to be into the appropriate bronchus the effect seems significant. Therefore, the use of these enzymes is recommended if the instillation can be made directly into the bronchus of a known area of bronchial obstruction. Otherwise, the risk of an adverse reaction is too great to justify random instillation.

ACKNOWLEDGMENTS: We wish to thank the staff at the Municipal Contagious Disease Hospital of Chicago for their cooperation and Mr. Joseph Seto for technical assistance.

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DIURETIC EFFECT AND LOCAL TOLERANCE OF A NEW TYPE OF MERCURIAL RECTAL SUPPOSITORY: A PRELIMINARY REPORT

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Accumulation of edema in the usual case of cardiovascular and other chronic disease is constant and gradual. It would seem logical to attempt interruption of this process by regular, small, repeated doses of mercurials[15]. In this manner it has been possible to remove excess fluid as rapidly as it collects, thus maintaining body weight at or near basal level.

The methods of administering mercurials present many practical problems. Parenteral injections require the services of trained personnel and of necessity impose certain inconveniences and discomfort for the patient. Oral diuretics promised for a time to fill the need of a convenient dosage form for maintenance but in extensive clinical trial have not proved nearly as reliable as parenteral mercury[15].

As an alternative to parenteral medication attempts have been made previously to develop a satisfactory mercurial suppository[19]. However, in all studies reported the diuretic effect has been inadequate or inconstant. Local irritation of varying severity has occurred in a high percentage of patients and tenesmus has seriously interfered with retention of the suppository[3-5,7]. Administration of mercurial diuretics by rectum, therefore, has been rarely used in recent years.

The absence of significant local irritation characteristic of subcutaneous injections of mercaptomerin sodium (Thiomerin)[6,11-14,16,17] suggested the possibility that this compound might be well tolerated by the rectal mucosa, hence interest was revived in the use of a mercurial suppository for more convenient maintenance therapy. In Thiomerin the xanthine is replaced by a mercaptan, sodium thioglycolate (one of the monothials; these substances, in therapeutic doses, have been shown in laboratory animals to diminish the cardiotoxic and nephrotoxic effects of organic mercury while preserving the diuretic action[1,8-10]). The significant reduction in irritating properties that characterizes thionated mercury is attributed to this structural modification[13,14].

Procedure. Suppositories were made up containing 0.5 gm. mercaptomerin sodium* (equivalent to 165 mg. mercury) in a cocoa butter base, for clinical study of the efficacy and possible irritating properties of this compound in long-term administration by rectum in the human.

Preliminary controlled pharmacological studies[2] were carried out using suppositories containing the same amount of mercaptomerin sodium (the full human dose) in a series of 8 rabbits and 6 dogs. Six control animals (rabbits) received suppositories containing no mercury. All were sacrificed in 24 to 72 hours and the kidneys and rectum of each were removed for histological study. Microscopic examination of the kidney sections showed that a significant mercurial effect was obtained by the Thiomerin rectal suppositories tested. Examination of the rectal tissue failed to demonstrate significant irritation in the treated animals. The only inflam-

*Rectal Suppositories, Thiomerin Sodium, mercaptomerin sodium, were supplied through the courtesy of Wyeth Laboratories, Philadelphia, Pa.

TABLE 1.—DIURETIC EFFECT OF THIOMERIN SUPPOSITORIES FOR MAINTENANCE IN OUTPATIENTS

Diagnosis	*Patient*	*Age*	*Parenteral Mercury Previously*	*Thiomerin Dose*	*Suppositories Period of Observation*	*Diuretic Response to Suppositories*	*Parenteral Diuretics Required*	*Proctoscopic Evidence of Irritation*	*Complaint of Side Effects from Suppositories*
Arteriosclerotic heart disease	J. S.	74	2 cc./3rd day	1/2nd night	3½ mos.	Adequate: Dry wt. on supp. Gained 9 lbs. in control periods	0	None in 3½ mos.	0
	A. W.	70	0	1/2nd night	3 mos.	Adequate: Edema gradually eliminated, dyspnea relieved; edema returned in control period	0	None in 3 mos.	0
	M. C.	68	2 cc./2nd wk. did not control	1/2nd night	1 mo.	Adequate: Supp. potentiated injected dose, with control of edema	2 cc./2nd wk.	—	0
	W. D.	67	2cc./wkly.	1/2nd night	1 mo.	Adequate: Lost 4 lbs. in 1 mo. Edema eliminated	0	—	0
with hypertensive cardiovascular dis.	A. H.	58	Sporadic, every 2 to 3 weeks	1/2nd night	6 wks.	Adequate. Wt. stable, no significant diuresis	0	—	0
	A. R.	54	2cc./wkly.	1/2nd night	6 mos.	Adequate. Small initial wt. gain. After 1 injection, lost 10 lbs. in 1 mo. on supp.	Single inj. 2 cc.	None in 3 mos.	0
with diabetes mellitus	M. C.	64	2cc./wkly. or biwkly.	1/2nd night	6 wks.	Adequate. Wt. loss of 6 lbs. with supp. Good potentiating effect	Spread out to inj./2 wk.	—	0
	A. E.	65	2 cc./10 days to 2 wks.	1/2nd night	1 mo.	Ineffective. Edema increased	Injections resumed	—	0
Hypertensive cardiovascular dis.	A. B.	50	2 cc./3rd or 4th wk.	1/2nd night	5 wks.	Adequate. Wt. stable	0	—	0
Rheumatic heart disease	H. S.	42	2 cc./2nd wk.	1/2nd night	6 wks.	Adequate. Lost 8 lbs. and peripheral edema	Single inj. 2 cc.	—	0
	F. K.	36	Sporadic	1/2nd night	2½ mos.	Adequate. Lost 7 lbs. and dyspnea in 2 wks. Wt. remained stable afterward	0	—	0
	H. P.	64	2 cc./2nd wk.	1/2nd night	3 mos.	Adequate. Wt. approx. constant	0	None in 3 mos.	0
	L. F.	54	2 cc./twice weekly	1/2nd night	6 wks.	Ineffective. Edema increased	Thiomerin inj. required 3 times wkly.	None in 6 weeks	0
Total patients 13						Adequate: 85% (11 of 13) Ineffective: 15%			

matory reaction occurred in the rectal mucosa of a control animal sacrificed at 24 hours.

Local tolerance studies[18] were then performed in 7 human subjects, all of whom were examined at the beginning of the trial. Suppositories were inserted nightly for 2 weeks. Examination at the end of this time revealed no evidence of primary irritation.

These suppositories were administered to 25 patients suffering from cardiovascular and other chronic diseases associated with generalized edema. In some cases 2 or more factors contributed to the edematous state. The series was divided into 2 groups: 13 ambulatory outpatients examined in the clinic at intervals of 1 to 4 weeks (Table 1), and 12 acutely or chronically ill patients hospitalized for a diversity of edematous conditions (Table 2).

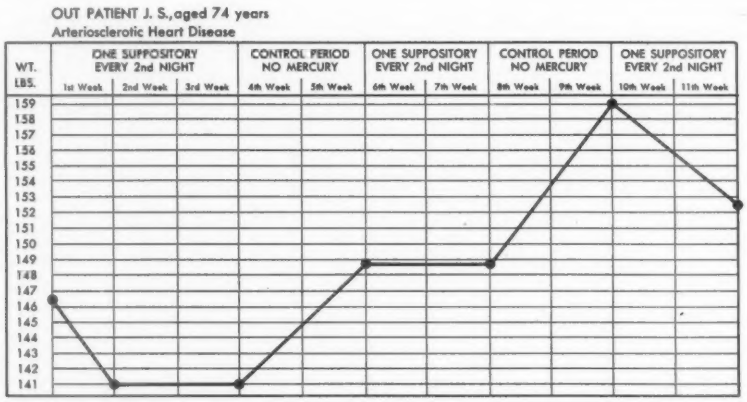

Fig. 1.—Weight graph of a patient who was treated intermittently with suppositories.

Diuretic therapy supplemented other measures required in each individual case, as bed rest, digitalization, dietary salt restriction, blood transfusions, and the like.

Of the *outpatient* group (in which the age range was 36 to 74 years), 8 had arteriosclerotic heart disease, 4 rheumatic heart disease, and 1 hypertensive cardiovascular disease. All were in chronic congestive failure.

Each received one suppository, administered at bedtime every second night for 1 to 6 months. In most cases suppository treatment was interrupted at various intervals by control periods of about 2 weeks, in which no diuretic was used. The case of a 74-year-old man with mild peripheral edema at the start of maintenance therapy illustrates the plan of management. This patient had received 2 cc. of a mercurial parenterally every third day while under hospital care, and after discharge was followed in the clinic for 3½ months. The suppositories were used for about 3 weeks, then stopped for a control period of 2 weeks; treatment was resumed for 2 weeks, and again interrupted by an additional control period of 2 weeks, after which the suppositories were continued for 4 weeks longer.

All but one of the other patients also had been treated with mercurials in doses of 2 cc. parenterally, at intervals ranging from twice weekly to once every 3 or 4 weeks before receiving suppositories for maintenance. One 70-year-old man, with arteriosclerotic heart disease, had never received mercurials before.

For the *hospitalized* group the etiologic diagnosis was arteriosclerotic heart disease in 6 patients, 54 to 71 years old; rheumatic heart disease in 2 patients, 49 and 59 years old; and hypertensive cardiovascular disease in 3. One of the hypertensive patients had an underlying periarteritis nodosa, and one had portal cirrhosis. One patient had Hodgkin's disease with ascites. Ten received one Thiomerin suppository nightly for one week, and 2 received one nightly for 2 weeks.

A 57-year-old man with lymphatic leukemia and a 40-year-old woman with periarteritis nodosa were treated, in addition, with 25 mg. cortisone, the former 3 times a day, and the latter twice a day.

The daily fluid intake and output were recorded for all in this group.

Clinical indications of loss or retention of edema were recorded for each patient in both groups: variations in weight, degree of pedal edema, hepatomegaly, dyspnea or rales. Each patient was questioned as to the

TABLE 2.—DIURETIC EFFECT OF THIOMERIN SUPPOSITORIES IN HOSPITALIZED PATIENTS

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Diagnosis	*Patient*	*Age, yrs.*	*Thiomerin Dose*	*Suppositories Duration Treatment Weeks*	*Diuretic Response to Suppositories*	*Parenteral Diuretics Required*	*Complaint of Side Effects from Suppositories*
Arteriosclerotic heart disease with congestive failure lymphatic leukemia, on 25 mg. cortisone t.i.d.	R. K.	57	1 nightly	1	Adequate. Lost 8 lbs. & peripheral edema in 1 wk. Followed 5 mos. in clinic. Maintained on supp. only	0	0
	J. G.	69	1 nightly	1	Adequate. Lost 10 lbs. & peripheral edema in 1 wk.	0	0
	A. E.	63	1 nightly	1	Adequate. Lost 12 lbs. & peripheral edema in 1 wk.	0	0
	J. H.	67	1 nightly	1	Adequate. Lost 4 lbs. & peripheral edema in 3 days	0	0
	R. D.	71	1 nightly	1	Adequate. Lost 22 lbs. in 1 wk. 0.2 mg. digitoxin b.i.d. for first 3 days of supp. treatment, then daily maintenance dose of 0.2 mg. digitoxin	1 cc. I.M. at start	0
diabetes with nephrotic syndrome	A. W.	54	1 nightly	1	Ineffective. Resisted all diuretic treatment	2 cc. I.M. daily; no effect	0
Rheumatic heart disease with congestive failure	M. W.	49	1 nightly	1	Adequate. Lost 12 lbs. & peripheral edema in 1 wk.	0	0
	J. R.	59	1 nightly	2	Adequate. Lost 16 lbs. in two weeks	0	0
Hypertensive cardiovascular disease, with left ventricular failure	E. K.	55	1 nightly	1	Adequate. Lost 16 lbs. by 9th day	2 cc. initially	0
with periarteritis nodosa; received cortisone 25 mg. b.i.d.	H. K.	40	1 nightly	1	Adequate. Lungs clear, no dyspnea in 4 days	0	0 (had been sensitive to inj. mercurials)
with portal cirrhosis and ascites	J. I.	41	1 twice daily	1	Ineffective. Edema increased	Eventual diuresis from 2 cc./2nd day	0
Hodgkin's disease, ascites, anasarca	L. J.	17	1 nightly	2	Ineffective. Necropsy showed nodes obstructing inferior vena cava	0	0
Total patients 12					Adequate: 75% (9 of 12) Ineffective: 25%		

presence of rectal discomfort or other evidence of local irritation. Direct proctoscopic examinations were performed when possible.

Diuresis on rectal therapy was considered adequate when the patient was maintained at an approximately constant weight after initial diuresis, or continued to lose fluid.

Results. The diuretic response was adequate in 85% (11 of 13 patients) of the *outpatients* (Table 1).

Seven patients continued to lose weight when the suppositories were substituted for parenteral therapy. The most notable success was achieved in the 74-year-old male patient mentioned previously, who was maintained with the suppositories alone throughout subsequent observation for 3½ months. He gained approximately 9 pounds in each of the two 14-day control periods without diuretics.

One patient lost 8 pounds, with disappearance of peripheral edema in 6 weeks on the suppositories. Another lost 5 pounds and had less dyspnea in 2 weeks, with stabilization of weight during the subsequent 5 weeks of suppository treatment. A third lost 4 pounds in one month, with disappearance of pretibial edema. In one patient, a 54-year-old woman, there was a small weight gain initially, but after a single injection of 2 cc. Thiomerin and resumption of the suppositories she lost 10 pounds in one month. In another, edema was gradually eliminated on the suppositories alone, with relief of dyspnea. A 68-year-old woman with chronic congestive failure and massive edema was resistant to treatment with parenteral diuretics. Weight was not controlled until suppositories were used in conjunction with diuretics by injection, when a good potentiating effect was achieved, with gradual loss of fluid.

Four patients were maintained at approximately constant weight without parenteral therapy.

In most cases when the suppositories were stopped for the control periods edema returned to former peak levels.

The 2 individuals in whom fluid continued to accumulate despite use of the suppositories required injection of mercurials up to 3 times a week for control of their edema.

Of the *hospitalized* patients, 75% (9 of 12 patients) were satisfactorily maintained on the diuretic suppositories.

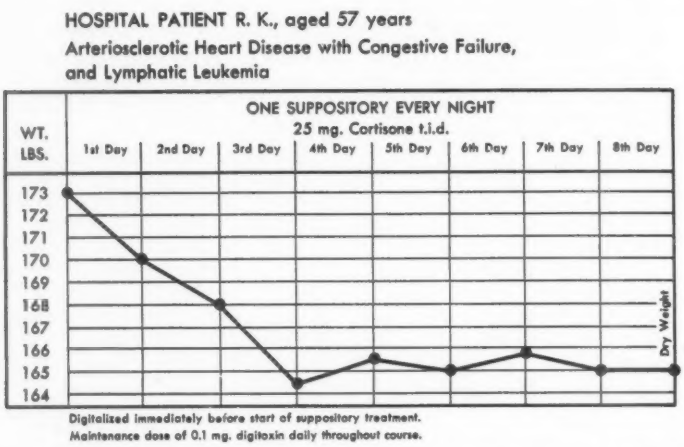

Fig. 2.—Weight response of a cardiac patient who was receiving cortisone.

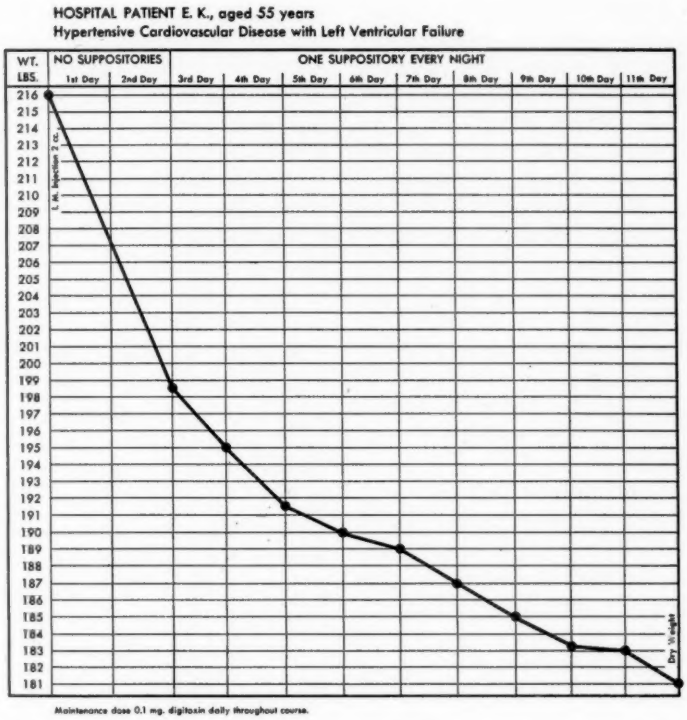

Fig. 3.—Progress of a patient who initially received parenteral mercury followed by suppositories.

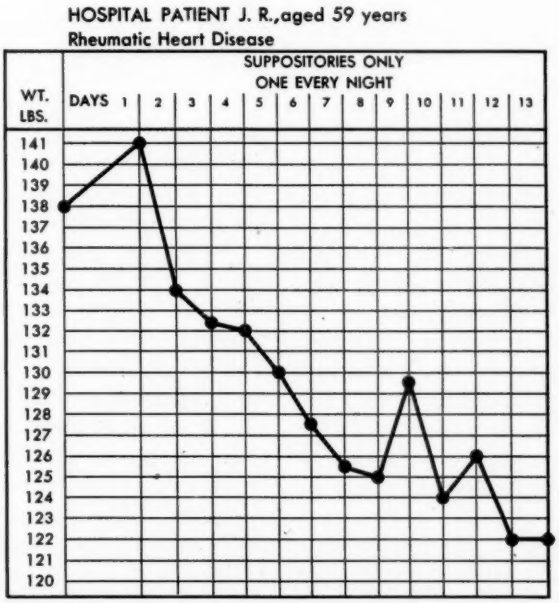

Fig. 4.—Weight chart of a patient who received suppositories and bed rest only.

One, the 40-year-old woman who received 25 mg. cortisone twice daily for treatment of periarteritis nodosa, was relieved of dyspnea with complete clearing of the lungs after 4 days on the suppositories. Another, a man who was treated with 25 mg. cortisone 3 times a day for lymphatic leukemia, lost 8 pounds, on rectal diuretic therapy, with disappearance of peripheral edema, during the week he was in the hospital. This patient was subsequently observed for about 5 months in the clinic, in which time he continued to use the suppositories regularly and was maintained edema-free without parenteral mercury.

All of the other 6 in whom the diuretic response was adequate experienced a remarkable loss of weight on the suppositories during their hospital stay: one lost 16 pounds by the end of the ninth day, and one lost 16 pounds in 2 weeks. Two others lost 12 pounds and one, 10 pounds, during one week in the hospital.

The peak of diuresis usually occurred on the second or third day after suppository therapy was started; hence for diuresis in acute conditions, such as pulmonary edema, a parenteral diuretic is recommended.

In 4 patients fluid accumulated so rapidly that parenteral treatment was required: (1) the 17-year-old girl with Hodgkin's disease; in this case necropsy demonstrated obstruction of the vena cava by enlarged nodes. (2) In the patient with portal cirrhosis diuresis was eventually achieved with 2 cc. Thiomerin by subcutaneous injection every second day. (3) A 71-year-old patient with congestive failure and (4) one with diabetes associated with the nephrotic syndrome and arteriosclerotic heart disease also required mercury by injection.

Among the patients proctoscoped no evidence could be seen of rectal irritation during continuous treatment for as long as 3½ months. No indication of mercurialism appeared, and no sign of sensitization. One patient (the woman with periarteritis nodosa) who had a definite history of sensitivity to mercurials by injection experienced an adequate diuresis on the suppositories with no untoward reactions. On direct questioning none of the patients complained of any local discomfort and none experienced symptoms of salt deficiency.

Summary. The diuretic effect and possible irritating properties of rectal suppositories containing 0.5 gm. Thiomerin, mercaptomerin sodium (equivalent to 165 mg. mercury) were tested in 25 edematous patients. Thirteen were outpatients seen in the clinic at intervals of 1 to 4 weeks, and 12 were hospitalized for a diversity of edematous conditions.

The outpatients received one Thiomerin suppository every second night for periods as long as 6 months, treatment being interrupted by control periods in which no diuretic was given. The hospitalized patients received one Thiomerin suppository nightly for 1 or 2 weeks.

The diuretic response was adequate (that is, the patient continued to lose weight or was maintained at an approximately constant level) in 85% of the clinic cases and in 75% of the hospitalized group.

Thiomerin suppositories controlled the tendencies to salt retention in 2 patients with an underlying cardiac deficiency who were receiving cortisone for other disease. This points to a broad field of usefulness.

These suppositories were particularly well tolerated. No evidence of untoward local or systemic disturbance was obtained, either on proctoscopic examination or direct questioning of the patients.

Thiomerin suppositories are adequate to maintain some patients edema-free and in others may be used as a supplement to parenteral diuretic therapy, so that injections will be required at less frequent intervals.

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COMPLEMENT COMPONENTS IN THE SERA AND URINES OF PATIENTS WITH SEVERE PROTEINURIAS*

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Our findings that serum complement levels are low in the nephrotic syndrome[4,5,6] which meanwhile has been well substantiated by numerous investigators[1,3,12] has found diverse explanations. While we believe that the fall in complement is due to an extensive antigen-antibody reaction with use of large amounts of complement, others have stated that it may be due to a lack of formation of certain complement components or to loss of complement or one of its components into the urine. The present investigation was undertaken to study quantitatively the amount of complement which could be possibly lost into the urine in the nephrotic syndrome and in other severe proteinurias which are not connected with a fall in serum complement. Since it is known that immune precipitates tie up certain components of complement while leaving others unchanged, the distribution of complement components in the sera of patients with the nephrotic syndrome may also give a clue as to whether or not it is probable that we are dealing with complement tie-up by specific immune precipitates.

Methods. SERUM. Sera of patients with the nephrotic syndrome taken and preserved in the way previously described[4] were examined for individual complement components according to the method of Ecker, Pillemer and Seifter[2]. In this method 4 reagent sera are prepared which do not have any complement activity by removing a different one of the 4 complement components from each of the 4 reagent sera. The only deviation from this method was made in the production of the reagent sera lacking C'3. Baker's yeast was weighed, washed 3 times with distilled water and once with saline, each time centrifuging for 5 minutes at 3,300 RPM and removing and discarding the supernate. The precipitated yeast was suspended in 10 times its weight of reagent serum and allowed to stand at room temperature for 30 minutes. The yeast was then removed by centrifugation for 5 minutes at 3,300 RPM and the supernatant serum reserved as the fraction lacking C'3. All reagent sera were tested for complementary activity and found to be devoid of it. All reagent sera were used in the tests in double amounts to insure an excess of all components except the one purposely eliminated. In each test the reagent sera lacking C'1 and C'2 respectively were combined in double amounts and a test for complementary activity was performed which always resulted in high titers. Since plasma volume determinations were not performed, plasma volume was estimated at 5% of body weight for the purpose of calculations.

URINE. Freshly collected urine specimens were analyzed for complement components

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according to the method of Seifter and Ecker[9]. Since preliminary experiments had shown that a full recovery of complement added to urine was not obtained when dialysis against a phosphate-saline buffer of pH 6.6 was carried out for 24 hours as suggested by Seifter and Ecker, a systematic study was initiated in which length of time of dialysis was varied. It turned out that 4-hours' dialysis at 2° C. against the phosphate-saline buffer using a mechanical stirrer in the dialysis bag according to the method of Reiner[8], resulted in recoveries above 95%. All urines were therefore dialyzed in this manner and then subjected to analysis for complement components according to the method used for sera with the following modification: In order to be able to detect small amounts of complement components in the urine, quadruple amounts of urine were used in each test. Again double amounts of reagent sera were used in each test as in the tests on serum.

The protein content of sera as well as of urines was determined by the Biuret method in each instance and the sera were examined for albumin/globulin ratios by precipitation with 22.5% sodium sulphate. Complement was determined in the way described by us previously[4], reading at the 50% hemolysis level. For the standard of one complement unit, complement was freshly prepared from commercial lyophilized guinea pig serum, diluted in steps of 5 from 1:5 to 1:50 and titration was carried out in the same manner as previously described[4] for the unknown serum. Guinea pig serum 0.1 cc. was mixed with 0.1 cc. inactivated human serum because guinea pig serum is known to have approximately twice the conplementary activity of human serum. The dilution of guinea pig C' which just produced 50% hemolysis was defined as one unit of complement (usually a 1:25 dilution). The number of units in the unknown serum was expressed as a multiple of this standard. Thus, if the serum under investigation produced 50% hemolysis in a 1:5 dilution its complement content was 5/25 = 0.2 units. With this method normal patients and patients with diseases other than those which specifically depress complement levels show an average complement content of 1.78 units with a range of 1.2 to 3.0 units.

Results. Ten sera of patients with the nephrotic syndrome in the edematous phase were examined for titers of individual complement components (Table 1). All sera had a diminished complement activity as is typical for the nephrotic syndrome. All sera showed C'2 to be the limiting component of activity. In all instances the titer of the test serum either equalled or very closely approximated the titer found for the limiting component. In 5 of them there was also a marked decrease of C'4. The degree of lowering of serum complement showed no relation to the hypoproteinemia or to the hypoalbuminemia or the globulinemia. The lowering of complement activity was also not in any discernible relation to the degree of proteinuria, as shown in Table 2. With clinical remission of the nephrotic syndrome complement returned to normal by a return of C'2 to normal. This was the case whether or not the hypoproteinemia and the hypoalbuminemia increased or the proteinuria decreased.

The serum complement values of 3 cases of Kimmelstiel-Wilson's syndrome with severe proteinuria were examined and found to be well within the normal range. One case of renal amyloidosis subsequent to long-standing suppuration of the descending colon also showed normal serum complement values in spite of a severe proteinuria (Table 2).

The content of individual complement components in the urine was determined in 7 cases with the nephrotic syndrome, 3 cases with Kimmelstiel-Wilson's syndrome and one case of renal amyloidosis. As is evident from Table 2 the excretion of complement components varied widely as to amount and the predominant component. In 3 of the 7 cases with the nephrotic syndrome C'2 was the predominant component excreted. The 4 other cases, however, showed other components to be predominant. There was no relation between the type of disease and the predominant complement component excreted. There was also no relation between the degree of proteinuria and

either the amount or the identity of the predominant component. Complement components were excreted in considerable amounts in Kimmelstiel-Wilson's syndrome and in renal amyloidosis, both diseases with normal serum complement levels, as well as in the nephrotic syndrome with lowered serum complement levels. Although it may appear from Table 2 that the percentage of excretion of complement into the urine is somewhat higher in the nephrotic syndrome than in the diuresis had occurred and complement levels had returned to within the normal range.

Discussion. The results described above for urine closely resemble in the general direction those obtained by Seifter and Ecker. These authors did not study the serum complement levels in the same patients at the same time, however, and their reports give no data as to weights of the patients or total urinary volume, so that it is, therefore, impossible to calculate from their data

TABLE 1.—COMPLEMENT COMPONENTS IN THE SERA OF 7 CASES WITH THE NEPHROTIC SYNDROME

Name	*Diagnosis*	*Complement Units*			
		C'1	*C'2*	*C'3*	*C'4*
E. L.*	NEPHROTIC	1.7	0.9	1.8	1.4
J. H.		1.2	0.6	1.1	1.7
before therapy					
J. H. after		1.9	0.7	1.4	1.4
partially successful					
therapy					
M. C.*		1.1	0.6	1.8	0.9
D. Y.		1.2	0.2	1.3	0.6
A. L.		1.0	0.36	1.1	1.0
before therapy					
A. L. after		2.0	0.64	2.0	1.9
partially successful					
therapy					
Mc. C.*		2.3	0.64	2.3	0.96
J. L.*		2.1	1.1	1.8	1.6
J. L.*		2.0	1.2	2.2	1.1

* adults

proteinurias of other origin, this impression is erroneous. The values are calculated on the basis of the estimated plasma volume multiplied by the complement content in units per cc. Since the complement content in the sera of nephrotics was low, the total complement content in the plasma was low and the percentage excreted appears unduly high as compared to the cases which had normal serum complement levels. There was no appreciable diminution of complement excretion in cases with the nephrotic syndrome after the percentage of the total serum complement which was excreted into the urine.

The irregularity with which we found the different complement components to be excreted into the urine is similar to the complete variability of the electrophoretic patterns in such urines, as described recently by Slater and Kunkel[11].

We have seen in one case of severe serum sickness that serum complement rose from 0.2 units per cc. to 1.4 units per cc. within 24 hours. Seltzer, Baron

TABLE 2.—THE LOSS OF COMPLEMENT COMPONENTS INTO THE URINE OF A CASE WITH RENAL AMYLOIDOSIS, 3 CASES WITH KIMMELSTIEL-WILSON'S SYNDROME AND 7 CASES WITH THE NEPHROTIC SYNDROME AND ITS RELATION TO THE TOTAL SERUM COMPLEMENT POOL

Name	*Diagnosis*	*Wt., Kg.*	*Assumed Plasma Volume, cc.*	*Compl. Units per cc. of Serum*	*Total C′ in Serum, Units*	*C′ Components in Urine, per cc.*				*Urine Volume in 24 hrs., cc.*	*C′ Excr. in 24 hrs., Units**	*Urinary C′ / Plasma C′ % 24 hrs.*	*Prot. in Urine, %*
						C′1	*C′2*	*C′3*	*C′4*				
Le.	renal amyloidosis	76	3800	1.3	4940	.04	0	0	0	1560	62	1.3	.66
Pa.	Kimmelstiel-Wilson Synd.	68	3400	1.7	5780	0	.1	.05	.1	1500	150	2.6	.68
Wa.	Kimmelstiel-Wilson Synd.	72	3600	2.1	7560	.11	.42	.42	0	600	252	3.3	.70
A. C.	Kimmelstiel-Wilson Synd.	58	2900	1.6	4440	0	0	.14	.06	616	86	1.9	.46
Sh.	glom. nephr. nephrot. synd.	53	2650	1.5	3975	.03	.06	.03	.03	2400	144	3.7	.15
Lo.	glom. nephr. nephrot. synd.	63	3150	1.1	3465	0	0	.22	0	1700	374	11.9	1.88
Ha.	glom. nephr. nephrot. synd.	20.5	1025	0.88	902	.05	.14	0	0	900	126	13.9	.88
D. Y.	nephrot. synd.	26.5	1325	1.0	1325	.03	.04	0	0	1400	56	4.2	.16
M. C.	nephrot. synd.	77.5	3850	0.65	2502	0	.025	0	.04	1300	52	2.0	.84
A. L.	nephrot. synd.	41.0	2050	0.64	1312	0	.05	.03	.07	700	49	3.8	.40
J. L.	nephrot. synd.	113	5650	1.0	5650	0	.15	.30	.05	650	195	3.4	.90

* Based on the value of the predominant component.

and Fusco[10] have reported that in guinea pigs a complete depletion of complement activity by the injection of an antigen-antibody complex is followed by a rise to normal complement levels within 20 hours. In patients with the nephrotic syndrome we have noted on numerous occasions that complement rose from one day to the next by at least one unit prior to diuresis, without a decrease in proteinuria or complement component excretion. It thus appears that the restoration of normal complement levels after depletion by immune precipitates can be very rapid. It seems probable then that losses of complement activity due to loss of one or more of its components into the urine of the magnitude of 1 to 14% per day can be well covered by the normal production of complement and should not be the cause for a lowering of serum complement levels. Moreover, in diseases in which serum complement levels are normal or high, but in which severe proteinurias are present, complement component excretions were found of a similar magnitude as in the nephrotic syndrome with lowered serum complement levels. Finally, in therapeutically induced remissions of the nephrotic syndrome, serum complement levels rise to normal values in a short period of time, while the urinary excretion of complement components persists or is even aggravated in some instances. It appears highly improbable, therefore, that the excretion of complement components into the urine in the nephrotic syndrome can be the cause of the lowered serum complement levels. The specific depression of C′2 and C′4 in the sera of patients with the nephrotic syndrome resembles closely the depression of complement components produced by specific immune precipitates, as seen by Pillemer and collaborators. The fact that the identical components are removed from rat sera in perfusion experiments through the isolated rat kidney when nephrotoxic sera were added[7], further supports the contention that the lowering of serum complement levels in the nephrotic syndrome is due to a specific antigen-antibody reaction.

Summary. 1) Serum complement levels are subnormal in the nephrotic syndrome but are normal in Kimmelstiel-Wilson's syndrome and in renal amyloidosis, although all 3 conditions show severe proteinuria,

2) The lowering of serum complement activity in the nephrotic syndrome is due to a low content of complement component C′2. In some cases C′4 is also depressed.

3) Certain complement components are excreted into the urine in small amounts in all severe proteinurias studied, irrespective of serum complement level, the hypoproteinemia or the degree of proteinuria.

4) The predominant complement component excreted into the urine differed and did not show any relation to the type of disease or the degree of proteinuria.

5) The amount of complement which could possibly be inactivated by this loss of one component is too small to explain the lowering of serum component in the nephrotic syndrome. An excretion of complement components of a similar magnitude does not lead to lowering of serum complement levels in Kimmelstiel-Wilson's syndrome or renal amyloidosis.

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INVESTIGATIONS INTO THE SITE OF COMPLEMENT LOSS IN EXPERIMENTAL GLOMERULONEPHRITIS*

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Serum complement is always lowered in acute and subacute human glomerular nephritis and in most cases with the nephrotic syndrome. While we have assumed that this lowering of serum complement is due to an extensive antigen-antibody reaction taking place in the kidney in which the glomerular surface has become antigenic, there is no direct evidence available that the kidney is a site of complement binding[6].

In the experimental production of acute glomerulonephritis or the nephrotic syndrome, or both, by nephrotoxic sera, serum complement also falls[2,10]. A marked difference, however, can be noted in the time of the occurrence of this fall in serum complement. When antiratkidney rabbit sera are injected into rats, complement falls immediately to very low levels, rises rapidly thereafter and returns to normal within 24 hours. With it, alterations in the kidney morphology can be noted almost immediately upon the injection of the serum. If antiratkidney duck sera or antirabbitkidney duck sera are injected into rats and rabbits respectively[7,10], no such immediate fall of complement occurs in most instances, and immediate morphologic changes of the glomerular apparatus are absent. Only after 5 to 10 days does serum complement begin to fall and simultaneously morphologic changes in the glomerular apparatus become apparent. In these experiments serum complement remains low in the rabbits when clinical healing does not occur.

In order to determine the role of complement and the site of its loss in experimental glomerulonephritis, isolated rat kidneys were perfused by recirculating rat serum containing complement, and the complement titers after addition of different nephrotoxic sera were measured.

Methods. Adult male rats of the Wistar strain, fed with Purina rat pellets and water *ad. lib.* weighing between 250 and 300 gm., were anesthetized with sodium pentobarbital intraperitoneally and when necessary small amounts of ether were given by inhalation. The peritoneal cavity was exposed and a fine glass cannula was tied into the aorta just below the diaphragm. Both the aorta and the cava inferior were ligated at the iliac bifurcation. An incision was made into the cava inferior distal to the entrance of the renal vein in all instances where only kidneys were perfused. An incision into the cava inferior just proximal to the entrance of the hepatic vein was made in all experiments

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where both kidney and liver were perfused. Perfusion with 500 to 1000 cc. of normal saline was then performed until the organs appeared completely free of blood and the return fluid was glass clear. The left kidney was then freed by carefully ligating and severing all vessels coming off between the renal artery and the point of insertion of the cannula into the aorta. The suprarenal vessels and the ureter were carefully ligated and severed. The kidney was then lifted out of its bed and the renal vein separated without ligation from the cava inferior at its junction.

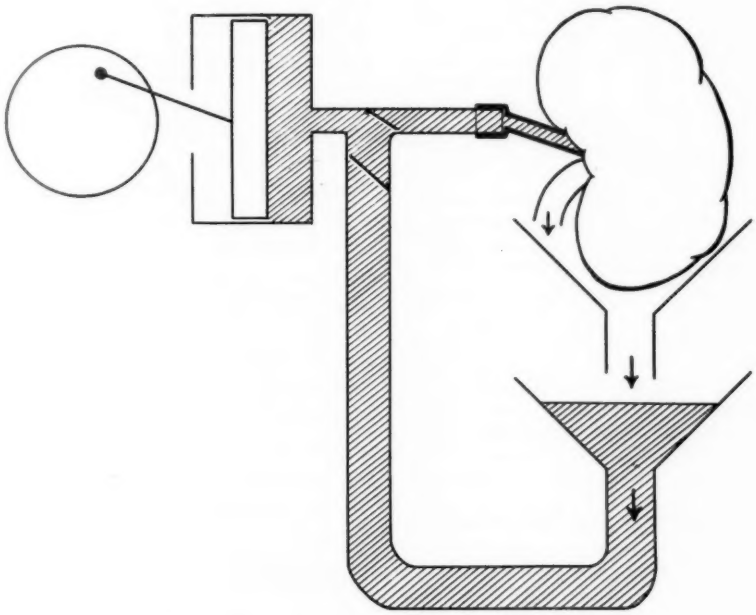

Fig. 1.—Schematic drawing of the perfusion set-up.

The preparation thus obtained was then placed into a small glass funnel which in turn was suspended over another small glass funnel serving as a reservoir leading to the inlet of a Sterling pipetting machine set to deliver 6 cc. per minute in 30 strokes. When the peripheral resistance of the kidney was high, however, only 2 to 4 cc. perfused through the kidney per minute. The cannula in the aorta was connected to the outlet of the pump (Fig. 1). The kidney was then perfused with 4 to 6 cc. of freshly prepared pooled rat serum gained the same day by decapitation of rats and separation of the serum at a temperature of 3° C. The entire perfusate thus recirculated approximately once every minute. Before the perfusion was started a specimen for complement determination was obtained. A specimen was again withdrawn 5 to 10 minutes respectively after the start of the perfusion. Immediately thereafter, the nephrotoxic sera or the control sera were added to the perfusate. Subsequent samples were withdrawn at intervals of 5 minutes. All specimens obtained were immediately placed in an ice bath.

For the liver perfusion, a cannula was introduced into the portal vein or hepatic artery and the liver lifted out of its bed. The hepatic veins were separated but not ligated at their entry into the cava inferior. In order to reduce the amount of liver tissue to be perfused, the left liver lobe was resected and discarded after carefully ligating the connecting hilus. The perfusion with recirculation and the tests were otherwise carried out in the same way as for the kidney.

At the end of each experiment an amount of the nephrotoxic serum aliquot to the amount used in the experiment was added to a sample of rat serum reserved before perfusion to test for anticomplementary activity of the nephrotoxic sera. This mixed sample

was left at room temperature for the same length of time as the experiment itself had lasted, in order to have comparable results.

Nephrotoxic sera were obtained from rabbits or ducks which were immunized against rat kidney by repeated injections of rat kidney homogenates. Nephrotoxic rabbit and duck sera were also obtained from other institutions*. All nephrotoxic sera were inactivated at 56° C. for 30 minutes before being used. All sera were shown to produce with great regularity a nephritic or a nephrotic picture, or both, when injected into rats. The complement determinations were carried out in the way described previously[6]. For the standard of one complement unit, complement was freshly prepared from commercial lyophilized guinea pig serum, diluted in steps of 5 from 1:5 to 1:50 and titration was carried out in the same manner as previously described[6] for the unknown serum. Guinea pig serum 0.1 cc. was mixed with 0.1 cc. inactivated human serum because guinea pig serum is known to have approximately twice the complementary activity of human serum. The dilution of guinea pig C′ which just produced 50% hemolysis was defined as a unit of complement (usually a 1:25 dilution). The number of units in the unknown serum was expressed as a multiple of this standard. Thus, if the serum under investigation produced 50% hemolysis in a 1:5 dilution its complement content was $5/25 = 0.2$ units. Since rat serum was shown to be much more thermolabile than human serum, all specimens were kept, and all titrations carried out, in an ice bath until the specimens were put into the water bath at 36° C.

When perfusates were analyzed for individual complement components the method of Ecker, Pillemer and Seifter[1] was used. The only deviation from this method was made in the production of the reagent sera lacking C′3. Baker's yeast was weighed, washed 3 times with distilled water and once with normal saline, and the supernate removed and discarded after centrifugation for 5 minutes at 3,300 RPM. The precipitated yeast was suspended in 10 times its weight of reagent serum and allowed to stand at room temperature for 30 minutes. The yeast was then removed by centrifugation for 5 minutes at 3,300 RPM, and the supernatant serum reserved as the fraction lacking C′3.

All fractions of the reagent sera, being always deficient in one complement component, were tested for complement activity and were found to be completely inactive. The reagent serum fraction devoid of C′1 and the reagent serum fraction devoid of C′2 were mixed before each test and shown to have high activity. To be sure of high titers if and when the test serum contained sufficient amounts of the missing components of the reagent serum, the reagent serum was always used in double amounts.

In a separate group of experiments it was found that when rat serum is fractionated according to the method of Pillemer and associates[1] the fraction devoid of C′1 did not produce a normal titer when added to the fraction devoid of C′2. The reasons for this behavior are at present under investigation. It was found, however, that the fractions of human complement can be used as reagent sera to test rat sera since the individual components of rat serum complement are able to replace components missing in human complement.

Results. Eighteen isolated rat kidneys were perfused with 4 to 6 cc. of fresh rat serum. An initial drop in complement titer of approximately 0.2 units, but occasionally up to 0.8 units was noted with great regularity during the first 5 minutes of perfusion. No further drop occurred thereafter if no nephrotoxic sera were added. This initial drop may be due to dilution of the perfusate with saline still retained in the glomeruli in spite of perfusing the kidney with air before starting the serum perfusion. There may also be an initial unspecific adsorption of complement. When 0.4 to 0.6 cc. of antiratkidney rabbit sera (ARKR) were added to the perfusate the complement titer in the next specimen obtained after 5 minutes of perfusion was always 0 (Fig. 2). Eight isolated rat kidneys were perfused with 4 to 6 cc. of rat serum and from 0.1 to 1.0 cc. of ARKR was added in increments of 0.1 or 0.2 cc. Samples for complement determinations were withdrawn 5 minutes after each addition of 0.1 or 0.2 cc. of ARKR. There was a direct relation

*We are greatly indebted to Dr. Beatrice Seegal and Dr. Walter Heymann for supplying some nephrotoxic sera.

between the amount of ARKR added and the fall in complement titers (Fig. 3). The slope of the fall differed slightly with different ARKR used. When plain rabbit serum was used instead of ARKR no fall in complement occurred. When 0.5 cc. of ARKR was premixed with 5 cc. of fresh rat serum, the effect of cortisone added to the perfusate before the addition of ARKR was investigated. In 3 experiments 5 mg. of cortisone acetate was added to the perfusate. Neither did the complement titer of the perfusing rat serum change when the cortisone was added, nor was the fall in complement titer

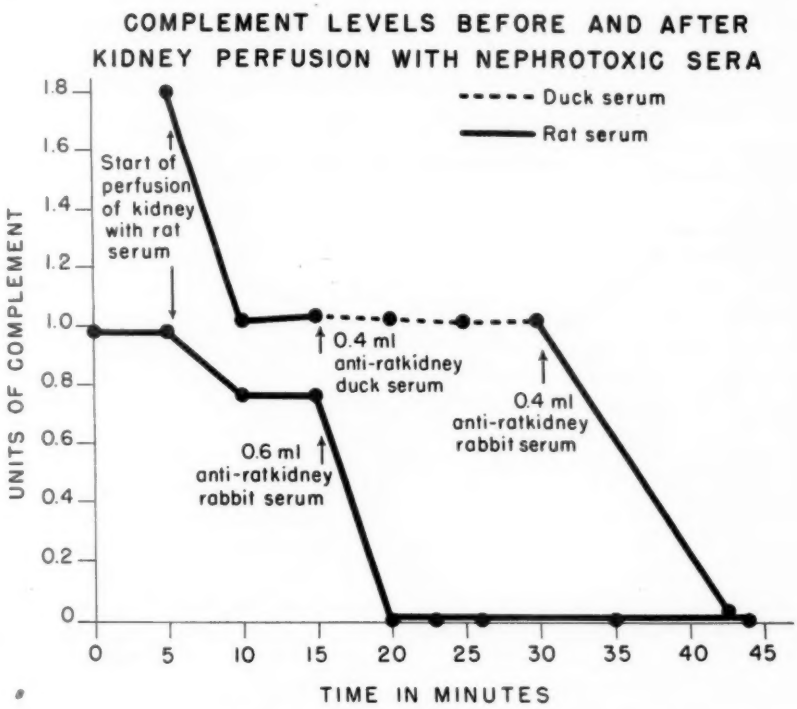

Fig. 2.—Complement levels before and after kidney perfusion with nephrotoxic sera. The lower curve shows the effect of 0.6 cc. antiratkidney rabbit serum. The upper curve shows the effect of antiratkidney duck serum in a different experiment followed by the effect of antiratkidney rabbit serum.

the complement titer which was normal after mixture but before perfusion, fell to 0 after one to 3 passages through the isolated kidney. This result was constant in all 5 experiments of this type.

Since cortisone produces diuresis in a high percentage of cases with the nephrotic syndrome preceded by a rise of the low complement titers to normal, upon addition of the ARKR and circulation through the kidney prevented.

Sera of ducks immunized against rat kidney by repeated injections of rat kidney homogenates were used instead of ARKR. These sera (ARKD) were known to produce with great regularity a nephritic or nephrotic picture in the recipient rat after several days. None of the 3 ARKD sera used

produced a fall in complement when added to the rat serum and perfused through the isolated rat kidney (Fig. 2).

In order to see whether the fall in complement when ARKR was used in the perfusate is completely or partially specific to kidney perfusion, parts of the liver of 5 rats were perfused. Upon addition of ARKR to the serum perfusate as perfusate and the same ARKR, the ratio of complement disappearance of $\frac{\text{liver}}{\text{kidney}}$ was 0.31 per gm. wet weight of tissue perfused.

In order to see which components of complement specifically led to the reduction of complement titer in these experiments, the perfusate of 6 kidney perfusions with rat serum and ARKR

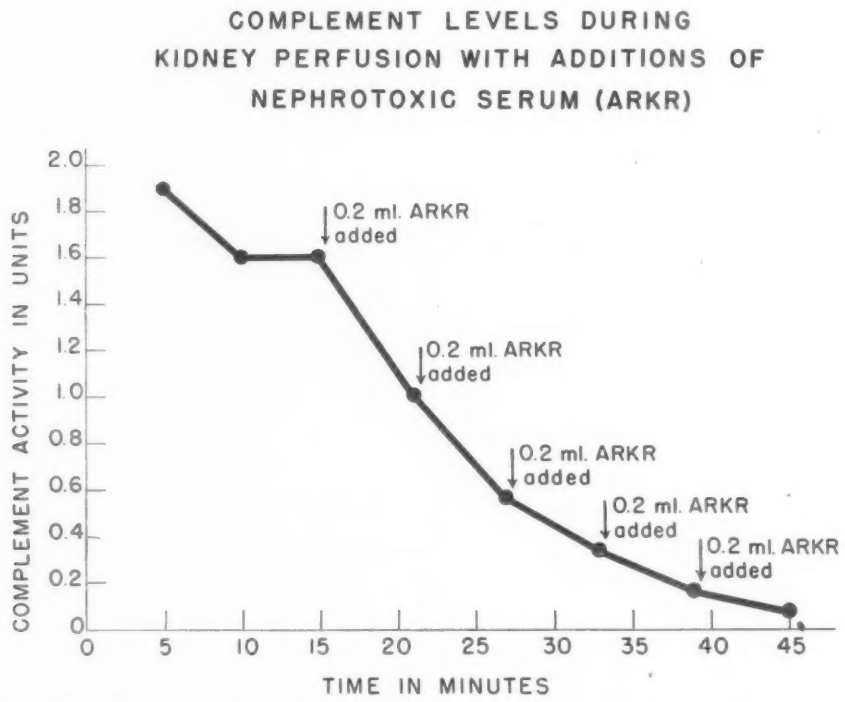

Fig. 3.—The effect of antiratkidney rabbit serum on the complement levels before and after kidney perfusion with nephrotoxic serum added in increments of 0.2 cc.

ing the liver a marked fall in complement titer could again be noted. On a weight by weight basis, however, the loss of complement activity was considerably less in the liver perfusions than in the kidney perfusions. If, for example, perfusions of the isolated kidney and isolated liver of the same rat were performed on the same day using the same fresh pooled rat

were tested for individual complement components. In all sera the reduction of complement activity could be traced to component C'2 as the limiting factor. In all cases there was also a marked reduction of component C'4 while C'1 and C'3 appeared unaltered.

Discussion. It has been suggested that the lowering of serum complement, especially in the nephrotic syn-

drome may be due to loss of complement or one of its components into the urine of patients with severe proteinurias[13], or that the lack of formation of complement or one of its components may be the reason for the low values found[3]. We were able to show that the loss of complement into the urine is not the reason for the lowering of complement in acute glomerulonephritis or the nephrotic syndrome[8]. It appeared necessary to localize at least in experimental nephritis the site of complement loss and to obtain an approximation of the amounts of complement activity which can be lost at such a site. There appears to be a considerable difference between the behavior of complement when mammalian or when avian nephrotoxic sera are given to mammals. When mammalian sera are injected an immediate fall in complement occurs together with an immediate appearance of disease followed by a rapid rise of complement activity to normal[2,7]. This behavior is exactly mirrored in the experiments described above where large amounts of complement are lost in one passage through the kidney when ARKR is added to the serum perfusate of isolated kidneys. In this type of experimental nephritis the antibody reacts immediately with the glomerulus or one of its parts and cellular destruction occurs in the presence of, and under the use of, complement, thus reducing the complement level of the circulating blood. Since 0.3 to 0.4 cc. of nephrotoxic sera passing through one kidney can remove all complement activity from 5 cc. of rat serum it is well understandable that the complement titer in the whole animal will fall to 0 in rats injected with 1.0 to 1.5 cc. of nephrotoxic sera. If one considers in addition that considerable amounts of complement are removed by the liver and probably by other vascular organs not yet studied, such a fall in complement is explained by immunological reasons alone and does not require the assumption of loss of complement components into the urine or lack of formation of one or all of the complement components. Since, in human glomerulonephritis and the nephrotic syndrome complement remains low during the active disease, the experimental glomerulonephritis produced by mammalian nephrotoxic sera appears immunologically only remotely related to the human disease. It cannot be used for therapeutic trials which attempt to suppress autoantibody formation. In our and Ogawa and Sato's[7,10] experiments, nephrotoxic sera produced in ducks did not lead to an immediate fall in serum complement. Since Pressman, Korngold and Heymann[11] have shown that these duck sera localize immediately in the glomerular apparatus, it appears that this union of antigen and antibody is not connected with fixation of complement.

Izumi[4] has described similar findings in *in vitro* experiments using mammalian antigen and avian antisera. It appears that cell destruction in experimental glomerulonephritis is dependent upon the fixation of complement or one of its components. For in the use of avian nephrotoxic sera the kidney remains morphologically unaltered until several days later when autoantibodies to duck serum are formed by the recipient animal, as was so clearly demonstrated by Kay[5]. When these antibodies start to join with the fixed antigen in the kidney, complement levels fall, accompanied by cellular destruction. The complement levels then remain low as long as antigen is available and antibodies are produced in a similar fashion, as seen in the human disease.

The lowering of serum complement when liver was perfused with nephro-

toxic sera is in good agreement with the findings of Pressman and Keighley[12], using radioactively labelled nephrotoxic sera and with the findings of Masugi[9] who always found damage to the vascular tree of the liver when nephrotoxic sera were injected. It appears from these and our findings that at least nephrotoxic sera gained from whole kidney homogenates are directed more against capillaries throughout the body than only specifically against glomerular tissue.

The removal of predominantly complement component C′2 and to a lesser extent of component C′4, by the perfusion of rat kidneys with nephrotoxic sera, is identical with the pattern found by us when sera of patients with acute glomerulonephritis or the nephrotic syndrome were analyzed for the limiting components of complement activity[8].

Summary. 1. Isolated rat kidneys were perfused from artery to vein with recirculation, with freshly prepared rat serum. When small amounts of antiratkidney rabbit serum were added to the perfusate the complement titer of the perfusate fell to 0 after passage through the kidney.

2. When antiratkidney rabbit sera were added in small increments to the kidney perfusate, the fall of complement activity after passage through the kidney paralleled the amount of nephrotoxic serum added.

3. One passage through the kidney often sufficed to remove all complement activity in this experiment.

4. Cortisone added to the perfusate in large amounts did not prevent the fall in complement activity.

5. Antiratkidney duck sera did not produce a fall in complement activity of the perfusate circulating through the kidney.

6. When antiratkidney rabbit serum was added to rat serum perfusing an isolated rat liver, complement activity also fell. The complement loss, however, was much less on a weight by weight basis when liver was perfused than when kidney was perfused.

7. The lowering of complement activity after the addition of nephrotoxic sera to rat kidney perfusions was primarily due to removal of component C′2.

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ON THE FORSSMAN-LIKE BEHAVIOR OF COLD HEMAGGLUTININS FOR SHEEP ERYTHROCYTES*

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Cold hemagglutination was originally described by Landsteiner[8] in 1903. During the next 40 years most of the interest in this phenomenon was limited to laboratories where it was often dealt with as a curiosity. In the past decade, however, there has been an increasing interest in cold hemagglutination of human erythrocytes not only in the laboratories employing it as an ancillary diagnostic procedure for primary atypical pneumonia[10], but also because the presence of these antibodies in high titer may rarely be implicated in the clinical symptomatology of a patient[6,7,13]. More recently, it has been pointed out that cold agglutination of sheep erythrocytes may be a significant factor in causing so-called false-positive heterophile agglutination tests for infectious mononucleosis[16]. In view of the latter observation, it became of interest to determine whether or not cold agglutinins for sheep red blood cells behave as Forssman antibodies.

Forssman antibodies, it will be recalled, belong to a large class of heterophile antibodies. The latter are defined as antibodies which are capable of reacting with antigens that are unrelated to those which stimulated their production[5]. Forssman antibodies constitute a subgroup of heterophile antibodies, and are differentiated on the basis of absorption characteristics. Forssman-type heterophilic antibodies are absorbed by suspensions of guinea-pig kidney but may or may not be absorbed by suspensions of boiled beef erythrocytes. The heterophile antibodies which are induced by infectious mononucleosis, on the other hand, are not absorbed by guinea-pig kidney suspensions, but are removed by absorption with boiled beef erythrocytes. These characteristics have been utilized by Davidsohn to devise a differential absorption test for the diagnosis of infectious mononucleosis[1].

The purpose of this report is to present the results of pertinent absorption studies of cold agglutinins for sheep erythrocytes. In addition, certain implications of the findings will be discussed.

Material and Methods. SOURCE OF SERA. The sera used in this study were selected from 7,000 specimens collected from over 2,200 patients and normal subjects. The patients represent a wide variety of clinical material, including infectious mononucleosis, leukemia, lymphoma, collagen disease, serum sickness after tetanus antitoxin and due to penicillin therapy, acquired autoimmune hemolytic disease, and acute and chronic infectious diseases. All of the sera were subjected to the

*This study has been supported by the Hematology Research Fund, Temple University School of Medicine and Hospital.

heterophile agglutination procedure as performed in this laboratory[14,15]. The presence in a serum of both cold and warm agglutinins for sheep erythrocytes is determined by this method. Sera in which were detected cold agglutinins for sheep red blood cells were selected for absorption tests.

Absorption of the sera with guinea-pig kidney suspension or with boiled beef erythrocytes was performed as recommended by Davidsohn[1]. The technique was modified slightly by adjusting the volume of serum to 0.3 cc. and the amount of guinea-pig kidney-suspension or beef cell antigen to 1.125 cc., in order that the dilution of the absorbed serum specimen would be the same as that employed in the tests with unabsorbed sera.

Both absorbed and unabsorbed specimens from a given patient were tested simultaneously against the same erythrocyte suspension in order to eliminate the agglutination variability of cells from different sheep[15].

Results. The results of the absorption studies with sera which exhibited cold agglutinins for sheep erythrocytes are given in Tables 1 and 2. As can be seen in Table 1, unabsorbed sera from normal subjects, and from patients with lupus erythematosus, syphilis, idiopathic thrombocytopenic purpura, multiple myeloma, leukemia, cat-scratch disease, and serum sickness had cold agglutinins which were removed upon absorption with guinea-pig kidney suspensions.

Tests on sera from patients with infectious mononucleosis are shown in Table 2. Inspection of the results on the unabsorbed specimens reveals that the titers observed after overnight refrigeration at 4° C. are often reduced upon warming the tubes to 37° C. After absorption with guinea-pig kidney suspension, the titers generally equalled or were slightly less than those of the warm readings on the respective unabsorbed sera.

Discussion. The cold agglutinins present in the sera tested in this study behaved as Forssman antibodies in the absorption tests. The sera exhibited cold agglutination in dilutions up to

TABLE 1.—TESTS ON SERA WITH COLD AGGLUTININS FOR SHEEP ERYTHROCYTES

	Unabsorbed Titers After		*Absorbed with*	
Serum Source	*Overnight 4° C.*	*2 hrs. 37° C.*	*G. Pig Kidney Overnight 4° C.*	*Beef RBC Overnight 4° C.*
1. Syphilis	1:32	0	0	0
2. Lupus erythematosus	1:16	0	0	1:16
3. ?Lupus erythematosus	1:32	0	0	1:32
4. ?Lupus erythematosus	1:64	0	0	1:32
5. ?Lupus erythematosus	1:32	0	0	1:16
6. Lupus erythematosus	1:16	0	0	1:8
7. Idiopathic thrombocytopenic purpura	1:64	0	<1:16	—
8. Idiopathic thrombocytopenic purpura	1:64	0	0	1:32
9. Idiopathic thrombocytopenic purpura	1:32	0	0	1:16
10. Idiopathic thrombocytopenic purpura	1:32	0	0	1:32
11. Mother of purpuric infant	1:32	0	0	1:16
12. Serum sickness (T.A.T.)	1:64	0	<1:16	—
13. Multiple myeloma	1:64	0	0	1:64
14. Chronic granulocytic leukemia	1:32	0	0	1:32
15. Lymphocytic leukemia	1:32	1:8	0	1:32
16. Cat-scratch disease	1:32	0	0	1:32
17. ?Histoplasmosis	1:32	0	0	1:32
18. Neurasthenia	1:32	0	0	1:64
19. Normal	1:128	0	<1:16	—
20–24. Normals	1:64	0	<1:16	—
25–32. Normals	1:32	0	0	1:32
33, 34. Normals	1:32	0	0	1:16
35–37. Normals	1:16	0	0	1:16

1:128 in tests with the particular sheep cells employed. In other studies[16], cold agglutinin titers of 1:256 have been obtained in tests with random lots of sheep cells. Furthermore, since the titer has been shown to depend on both the specific serum specimen and the particular lot of sheep cells used, it is likely that even higher cold agglutinin titers may occasionally be encountered. Obviously, such titers are of sufficient magnitude to be misleading if their true nature is unrecognized. In this connection, the finding that cold agglutinins for sheep erythrocytes are absorbed by guinea-pig kidney suspension raises important questions with reference to the heterophile agglutination test for infectious mononucleosis. The original laboratory test described by Paul and Bunnell[9] called for reading the results after overnight refrigeration in the icebox. Similarly the results of tests employed by Davidsohn in 1929[2], 1933[3], and 1935[5], were all read after overnight refrigeration. In all of these tests, therefore, the cold agglutination phenomenon was undoubtedly a factor in many of the test results. It was during this period of investigation that Davidsohn introduced the guinea-pig kidney suspension absorption test for use in the "specific" diagnosis of infectious mononucleosis. Later, the Davidsohn procedure was modified to reading of the test after only 2 hours of incubation at room temperature. However, a number of laboratories[11,12] purporting to use the latter Davidson technique have, nevertheless, placed the tubes in the refrigerator overnight before reading the test results.

TABLE 2.—TESTS ON SERA FROM PATIENTS WITH INFECTIOUS MONONUCLEOSIS

		Unabsorbed		*Absorbed with G. Pig Kidney*	
Patient	*Serum*	*Titer After Overnight 4° C.*	*Titer After 2 hr. @ 37° C.*	*Titer After Overnight 4° C.*	*Titer After 2 hr. @ 37° C.*
S	49–1507	1:256	1:64	1:256	1:64
	49–1605	1:128	1:128	1:32	1:32
	49–1649	1:64	1:16	1:32	1:32
	49–1690	1:64	1:32	1:16	1:16
	50–24	0	0	1:8	0
	50–255	1:8	0	0	0
P	50–58	1:1024	1:256	1:1024	1:256
	50–212	1:32	1:16	1:8	0
	50–250	1:64	1:16	1:8	0
L	49–210	1:1024	1:512	1:256	1:256
	49–248	1:128	1:128	1:256	1:128
	49–529	1:128	1:64	1:32	1:32
H	52–321	1:512	1:128	1:128	1:64
	52–334	1:1024	1:256	1:512	1:256
	52–335	1:1024	1:512	1:512	1:512
	52–356	1:64	1:16	1:32	0

In retrospect, therefore, it appears that cold agglutinins may well have been responsible for much of the early confusion with regard to results of the Paul-Bunnell test. The guinea-pig absorption test apparently removes both cold and warm agglutinins of the Forssman type. However, cold hemagglutination is readily dispelled by rewarming the test serum-cell mixtures after overnight refrigeration. This is both more simple and more economical than the guinea-pig absorption procedure. It seems logical, therefore, that results of heterophile agglutination tests which include overnight refrigeration

in the procedure should be read only after a suitable period of further incubation at 37° C.

This procedure is followed in this laboratory and in thousands of tests performed over the past 7 years, warm agglutinins due to causes other than infectious mononucleosis have been infrequently detected. Furthermore, it has been extremely rare to observe a significant change in titer of such agglutinins in tests on successive serum specimens. Antibodies present in nonchanging titer are, of course, considered to be unrelated to a given patient's present illness. In contrast, there is a dynamic antibody response in infectious mononucleosis which can be detected by testing appropriate serum specimens under standard conditions[15]. Whenever unexplained warm agglutinins have been encountered in tests in this laboratory, the sera have been subjected, of course, to the guinea-pig kidney absorption procedure. On the basis of experience and the findings noted herein, however, it appears that the indications for absorption of sera with suspensions of guinea-pig kidney can be greatly reduced by simply eliminating the cold hemagglutinin factor.

Summary and Conclusions. Absorption tests were performed on human sera containing cold hemagglutinins for sheep red blood cells. The resulting absorption patterns indicate that such cold hemagglutinins behave as Forssman antibodies.

Cold agglutinins for sheep erythrocytes may be removed by absorption with guinea-pig kidney suspension or dispelled by warming to 37° C. In the interests of economy and simplicity, it is recommended that the latter procedure be followed in routine heterophile agglutination tests.

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PROGRESS
OF
MEDICAL SCIENCE

OTO-RHINO-LARYNGOLOGY

UNDER THE CHARGE OF

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RECENT TRENDS IN NASAL MEDICATION

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THE use of nasal medication is both widespread and popular. Mounting attention in recent years to nasal physiology, histopathology, and bacteriology has induced many clinicians to employ nasal medication in a sound, rational manner. This is all to the good; in an era that is still characterized by undisciplined usage of antibiotics there is greater need than ever for intelligent therapeutics.

Ever since Bordley[1] first observed that nasal sprays of cortisone result in a slow but definite regression of nasal polyps, interest among rhinologists and allergists in the intranasal application of the drug has risen. Dill and Bolstad[3] used cortisone in a 1:4 dilution (1 cc. Cortone acetate to 4 cc. of physiologic sodium chloride solution) as an intranasal spray 4 times daily in a group of 25 patients with allergic rhinitis. They found that the substance produces a more or less temporary effect, sustained in proportion to the length of treatment. Use of cortisone in 1:4 dilution lessened the amount of nasal discharge and decreased the edema of the nasal mucous membrane. Relief of mild asthmatic symptoms, such as wheezing, cough, and tightness of the chest, was noted by many patients, although severe asthmatic attacks were unaffected. Some nasal polyps were seen to shrink, others to disappear temporarily. In general, use of the diluted cortisone nasal spray produced temporary relief from the symptoms of allergic rhinitis. Of 25 patients thus treated, 12 (48%) showed pronounced improvement; 6 (24%) patients had some degree of improvement, but were classed as doubtful; while the remainder (28%) evinced no improvement and were considered failures. Wall and Shure[12] injected from 2.5 to 25.0 mg.

of cortisone in suspension submucosally in the inferior turbinates of 65 patients, 52 of whom had allergic rhinitis and 13 of whom had vasomotor rhinitis. Eighty per cent of the patients with demonstrable allergy responded favorably to the procedure, obtaining relief for periods lasting from 6 weeks to 10 months. The most satisfactory response was elicited in patients with allergic rhinitis; 12 of 13 patients with vasomotor rhinitis did not obtain benefit from the injections. Severe, immediate, unexplained constitutional reactions occurred in two patients.

To have maximum therapeutic value, a drug should be absorbed rapidly and also be easily administered. In this connection the intranasal route has considerable merit, especially because of the vascularity of the nasal mucous membrane and because the nasal cavity lends itself readily to the administration of nasal medication by patients. The importance of the nasal mucous membrane as a route for drug administration when systemic effects are sought was recently demonstrated by Tonndorf and his associates[11]. They compared intranasal medication with hyoscine (scopolamine hydrobromide) applied as nose-drops or by means of nasal sprays with the oral and subcutaneous administration of the drug. Employing a relative decrease in the production of saliva as a criterion, hyoscine was shown by them to be more rapidly, completely, and uniformly absorbed after intranasal use than after oral administration. Nose-drops were found superior to nasal sprays. This was believed due to the fact that because of the relation of droplet size to surface tension, the fine droplets produced by a spray are confined to the site at which they impinge, thus resulting in poor "wetting" of the epithelium. Moreover, part of the drug may even be swept along into the nasopharynx by muco-ciliary action. On the other hand, the comparatively large drops dispensed by nose-drop instillation may spread to much wider areas and establish better contact with the epithelium of the nasal mucous membrane. The addition of a detergent (wetting agent) to the nasal hyoscine spray provided as effective a medium of drug administration as nose-drops, presumably by permitting penetration of the layer of mucus. Hyoscine was shown to be absorbed at a rate and to a degree more nearly resembling subcutaneous administration than oral administration. This appears to differ with the findings of some investigators who either found no apparent systemic effects after intranasal administration, or else attributed these effects solely to the swallowing of excess medication[9]. The intranasal absorption of atropine was also studied by Hyde and his associates[5], who used the depression of salivary secretion as a criterion. The addition of a detergent and of hyaluronidase to atropine nose-drops had no effect on the rate or degree of absorption.

Since the introduction of liver therapy by Minot and Murphy, the treatment of pernicious anemia has undergone numerous alterations in procedure. Monto and Rebuck[7] utilized crystalline vitamin B_{12} in isotonic sodium chloride solution, administering the substance intranasally in a small group of patients with pernicious anemia in relapse. A satisfactory hematological and clinical response was obtained, maximum reticulocyte increase occurring between the eighth and tenth days of treatment, and correlating with the severity of the anemia. Nasal instillation of crystalline vitamin B_{12} produced no objective or subjective evidence of toxicity or sensitivity at the local site. Nasal instillation of the substance resulted in significant amounts of vitamin B_{12} activity in the urine.

Intranasal medication has definite indications and purposes. If these are recognized and if the underlying nasal pathologic condition is properly evaluated and the medication employed judiciously, satisfactory clinical responses can frequently be elicited. Nasal vasoconstrictors, for example, occupy a commanding position among the various therapeutic measures employed by the general public for the relief of nasal congestion and the common cold. Although ensuing relief is temporary, overcoming the discomfiture caused by intranasal obstruction is welcome to distraught patients. For physicians to disregard the opportunity of producing palliation, when such effect is easily possible, is tantamount to complete therapeutic neglect.

The problem of producing new sympathomimetic amines is of great importance and has been slowly gathering attention in the pharmaceutical world. In recent years various nasal vasoconstrictors in combination with various sulfonamides[10], antibiotics[2,6,8], and antihistaminic drugs have been introduced. Some of the available combinations include such antibiotics as polymyxin B sulfate, gramicidin, and neomycin. While some forms of combined intranasal liquid medication have their ardent supporters, other investigators view combination liquid nasal solutions with considerable restraint. In any event, when a nasal vasoconstrictor is combined with any of the currently established antibiotics, antihistaminics or sulfonamides, one feature is certain: physiologic relief is obtained primarily by the shrinking component.

Fabricant[4] not long ago described the effect of progressively buffered solution of ephedrine on the nasal mucous membrane. In this investigation, the right nasal passages of 40 rabbits received measured instillations of a progressively buffered solution of 1% ephedrine hydrochloride in isotonic sodium chloride solution, the left nasal passages serving as a control, 3 times daily for a period of 30 days. The ephedrine preparations bore pH values ranging from 2.5 to 12.0. Each of the animals was killed at the end of 30 days, the turbinal structures removed, and sections prepared for histological study. At necropsy, gross purulent exudate was demonstrated in animals treated with solutions of ephedrine buffered to pH 3.0, 3.5, 4.0, 4.5, 7.0, 11.5, and 12.0. In numerous instances the microscopic sections revealed a similarity between the ephedrine-treated and the untreated nasal mucous membranes, with minimal histological changes. For the most part, the pathological changes in the respiratory nasal mucous membranes consisted of slight to considerable hyperemia; acute, subacute, and chronic inflammation; and varying degrees of suppuration. In general, highly acid and highly alkaline solutions are more likely to produce traumatic and suppurative nasal mucosa changes.

Finally, Fabricant[4] points out that in the human nasal cavity, the pH of nasal secretion *in situ* in clinically normal adult nasal passages ranges from approximately 5.5 to 6.5, whereas in infants and young children it ranges from 5.0 to 6.7. During acute rhinitis, acute sinusitis, and the more active phases of allergic rhinitis, the pH of nasal secretion is on the alkaline side, and then shifts back to the acid side when the state of clinical resolution is reached. It is during the time of acute nasal embarrassment that the nasal mucous membranes are inflamed or congested, the secretions are increased, and nasal breathing itself becomes more labored. Nasal alkalosis takes place in response to the act of hyperventilation (blowing off of carbon dioxide), and this presumably can explain

in part the reason for the alkaline nasal state during acute rhinitis, acute sinusitis, and the more active stages of allergic rhinitis.

In conclusion, intranasal treatment of the human nasal cavity by means of rational, physiological nasal medication requires not only treatment of mucous membranes (including ciliary action) but also treatment of nasal secretions (including the mucous blanket). Normal nasal secretion, including the mucous blanket, possesses a purposeful acid barrier against infection, for it has been established that acidity as reflected in low pH values is unfavorable to the growth of pathogenic bacteria and that nasal secretion possesses an acid composition when receding after allergic attacks.

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NODULAR VASCULITIS

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NODULAR lesions of the legs present difficult clinical problems. In previous reviews two processes[8,9] with nodular lesions, often involving the legs, have been described. The present compilation details information on a third type, not so well known, nodular vasculitis. It is the purpose of the account to compare nodular vasculitis with erythema nodosum, liponecrosis (Weber-Christian syndrome) and especially with erythema induratum, which is in reality probably tuberculous nodular vasculitis.

Nodular vasculitis is a designation for a number of nodular syndromes, the causation of which cannot be determined but the morphology of which reveals the group to be closely aligned to erythema nodosum and erythema induratum. It is a widely inclusive term embracing both acute and chronic processes, and it has been applied to cases of nodular phlebitis and periphlebitic indurations[43]. Erythema nodosum is an acute nodular process which, although it may be of idiopathic nature (specific disease) in some cases, may also be the result of tuberculous infection, rheumatic infection, an infection with Beta hemolytic streptococci, a nonspecific allergic reaction, and a nonspecific manifestation of various factors of toxic type or concurrent infections. It is not always possible to differentiate with certainty the idiopathic type from the symptomatic variety especially that due to tuberculous infection. Only after prolonged search in some instances is the tuberculous focus uncovered. Fat

necrosis, while not specifically involving the legs, localizes on the lower extremities often enough to be included in this discussion. The syndrome of Weber-Christian represents a spontaneous type of circumscribed panniculitis. Montgomery, O'Leary and Barker[45] have designated another clinical variant of spontaneous panniculitis, the nontuberculous form of erythema induratum, as nodular vasculitis. Thus the borderline between true idiopathic panniculitis and nodular vasculitis is indefinite. True erythema induratum has long been considered as being tuberculous in origin but only a few cases have had this background proven. Authorities have often insisted on the necessity of proving the tuberculous background of these cases[68] and some have even denied that tuberculosis has anything to do with this process at all. Sulzberger[65] has summed up the viewpoint as follows:

"Dermatologists must agree today with Brocq's idea of *reaction cutanée* in regard to many of the morphologic entities. For example, in nodose erythemas, in multiform erythemas, in sarcoid reactions and in similar conditions it has been proved that closely similar or almost identical clinical and microscopic pictures can be produced by different etiologic agents. It would be tempting to say that the same remark may apply to those conditions of the legs diagnosed as erythema induratum or papulonecrotic tuberculid and that they may have a different cause in different cases. Unfortunately, this has never been proved to be true. It does not seem that any typical case has ever been proved due to any etiologic agent other than the tubercle bacillus. Thus, a case of nodose erythema may be caused by a streptococcus, by the lepra bacillus, by the virus of lymphogranuloma venereum, by a drug such as bromide or iodide or salicylate and by one of many other etiologic agents. However, there are no authenticated and well studied cases of Bazin's disease with the typical clinical picture and histologic changes and no case of typical papulonecrotic tuberculid in which causation by an agent other than the tubercle bacillus has been proved. All typical cases in which extensive, thorough and proper examination have been made have been shown to be of undiscoverable cause or have eventually been shown to be of tuberculous origin. In these etiologic examinations the determination of macroscopic or microscopic changes must necessarily play a minor role. For since one starts by granting the premise that similar or identical changes must be the result of different causes, one can obviously draw no binding conclusion as to causation by means of mere morphologic factors and consider epidemiologic ones, immunologic reactions and particularly bacteriologic investigations. Proper bacteriologic investigation of a cutaneous lesion suspected of being a tuberculid may have to consist of many biopsies of abundant tissue from various lesions in various stages of development and involution and numerous animal inoculations with this material. It is sometimes necessary to inoculate as many as 10 guinea pigs from each specimen of tissue, and all the animals allowed to live until one is certain that tuberculosis will not develop in some one of them. Wherever one has been fortunate enough to have facilities for such studies, it has eventually been shown that in typical cases of Bazin's disease and papulonecrotic tuberculids tubercle bacilli are present, regardless of whether the histologic morphe presents banal perivasculitis or tubercle structures. In this manner, the lesions in Phillipson's own cases of 'Periphlebitis nodularis necrotisans' were shown to be tuberculids, and Phillipson himself

published this fact and withdrew his descriptive term in favor of Darier's etiologic designation."

While there is disagreement with respect to the possibility of multiple causes for the erythema induratum syndrome, all are agreed that most patients with this disease have unstable circulation (Chilblain), Michelson[43] summarizes this unstable circulatory state most succinctly. "Their blood vessels react in an abnormal manner to temperature changes; they nearly always have cutis marmorata which denotes a disturbance in the ability of the terminal vessels to accommodate themselves to physical stimuli. Very likely this results in a metabolic insufficiency which affects the chemistry of the tissues in the injured area."

On the other hand, O'Leary[48] called attention to the difficult diagnostic problem of nodular lesions of the leg. "The clinical picture may suggest thrombophlebitis, erythema nodosum, periarteritis nodosa, panniculitis or nodular vasculitis. Physicians who have had considerable experience with these conditions frequently differ in their clinical interpretation of these lesions. It is seldom that all are in diagnostic accord. The histopathologic study may be equally misleading if the biopsy is done with a skin punch, because in the removal of such a small piece of skin, the fundamental pathologic process may be missed and an erroneous diagnosis made on the secondary pathologic changes. Accordingly a wide excision of the nodule with the study of serial sections is more likely to result in accurate diagnosis. The term nodular vasculitis was coined to include the nodular lesions of the thighs as well as the lower parts of the legs in middle-aged adults, usually women. In patients with this condition evidence of tuberculosis as determined by histopathologic examination, animal inoculation studies and clinical surveys has been negative. The lesions are not as large or indurated as those of erythema induratum, and they recur in crops with periods lasting several months when the patients are free of the nodes. Hypertension in moderate degrees has been a most consistent observation in these patients." In nodular vasculitis too it is necessary for the patients to be examined by a physician who specializes in vascular diseases, for most of these patients will be found to have a serious vascular disturbance which has given rise to the affection.

Thus, since vascular changes are a basic factor of the nodular processes of the legs, a brief statement of the cutaneous circulation and cutis marmorata is in order.

By injection of dye, Renaut[57] demonstrated that each artery rising from the subcutaneous plexus supplies a conical section of skin, the apex of which is directed inward. Each cone is a unit, and each when injected, filled rapidly at first. However, as the adjacent blue areas approached each other, the spread of the dye was retarded and final fusion of the areas was relatively slow. The anastomatic channels at the periphery of each cone, while present, are relatively small, and it is in these regions that the circulation through the skin is slowest. In recognizing this, Renaut referred to the central "areas of maximum circulation" and peripheral "regions of reduced circulation." These regions of reduced circulation join one another in a network which presents angular "nodes" at the junction between three or more cones.

It is apparent that the number of arteries rising from the vascular plexus at the dermosubcutaneous border will determine the size of the cone. The relative size of the anastomatic channels will determine the degree to which the circulation is impeded in

the peripheral areas. In the normal skin this configuration cannot be seen.

Auspitz[4], in 1874, demonstrated that the network could be visualized by the application of a venesection band and he showed the relationship of the network to the lesions of measles and smallpox. Sir Thomas Lewis[36] traced the pattern on his own arm, and showed it to be constant within his ability to map it out. List, in studies of cutis marmorata demonstrated that the dark lines always occupied the same spots in any one individual. Lewis believed that any impairment of circulation tended to accentuate the reticular pattern. Adamson[1] discussed the relationship of livedo reticularis to other diseases such as urticaria, syphilis, parakeratoses variegata and Bazin's disease (erythema induratum).

The occurrence of this reticulation of the skin in some individuals and not in others defies explanation. It seems at times to be associated with xerosis of mild to moderate severity and is relatively frequent among mongoloids whose tendency to dryness of the skin is well known. Reticulation is an almost universal finding, post mortem.

The thermal response of the skin in this regard is also variable. Chilling will accentuate reticulation when present, and bring it out in some persons when it is not noticeable otherwise. Yet not everybody develops it even under severe exposure to cold. Conversely, it can sometimes be brought out by exposure to heat, but not all persons will develop the erythema ab igne.

Clinical manifestations of these cutaneous vascular phenomena have been divided into three stages[76].

1. Cutis marmorata, a transitory mottling due to imperfect vasomotor control.

2. Livedo reticularis idiopathica, differing in that the mottling does not disappear on application of pressure or warmth. Some cases of this seem to be nevoid in character and develop in families associated with other developmental abnormalities.

3. Livedo reticularis symptomatica, of which more will be said later.

Unna[70] was of the opinion that spasm of the veins would bring out the pattern, but Spalteholz as well as Ehrmann thought the difficulty lay entirely within the capillaries.

Krogh[33] has shown that not only are the arterioles and capillaries independently innervated, but the capillaries will respond to direct stimulation as well. In the presence of adequate oxygen tension the chief factor in maintaining capillary tone is a hormone which Krogh ascribes to the pituitary gland. Ebert[17] postulated that a reduction of circulation through these fringe areas by abnormal arterial spasm leaves these capillaries to be first affected by loss of oxygen tension and absence of the hormone.

Barker, Hines and Craig[26] comment that the reticular pattern often appears too coarse to be the stasis in fringe areas. This coarseness was studied by Williams and Goodman[76] in a case of idiopathic livedo reticularis. In their measurements they noted that at the periphery of the involved area fine lines of livedo radiate into and disappear in the normal skin. Just inside this the ratio of involved to uninvolved skin area is one to three. As one proceeds to the center of the affected part this ratio changes and reverses itself. Here small pale circles indicate the very center of the arterial cone; a similar situation was noted by Adamson in the case of erythema ab igne.

Those processes classified by Williams and Goodman as livedo reticularis symptomatica are deserving of further consideration. In this group they placed the cases in which livedo

was associated with, but not necessarily due to, other abnormal states and the general patterning was of a permanent nature. As examples they mention erythema ab igne, asphyxia reticularis multiplex of Unna, tuberculosis and syphilis. To this list Ebert added exposure to cold, arteriosclerosis, infectious diseases such as typhus and endocarditis, and toxic reaction following arsphenamine injections. Other additions to the list have been: alcohol[7], periarteritis nodosa[30,41,62], Weber-Christian Syndrome[18], erythrocyanosis[68] and sarcoid[1]. It will be noted that this list comprises a good differential diagnosis of the nodular vascular diseases affecting the extremities.

Barker, Hines, and Craig[26] reported cases ranging in severity from permanent discoloration through ulceration and cicatrization to gangrene necessitating amputation. Williams and Goodman sum up the situation thus: Cutis marmorata, mild as it is, is only the first of a long series, gradually increasing in extent and severity and constituting in the more severe cases a permanent disfigurement.

Nodular Vasculitis. The actual concept of nodular vasculitis has been long in developing. Since erythema induratum was first described in 1861[6], dermatology has passed through a series of evolutionary phases which give more basis for the grouping of numerous facts difficult to correlate into a nosologic class. Although many authors[11,15-17,20,24,42,46,53-56,61,75] had described the tuberculous origin of all cases of erythema induratum, it was not until 1945 that Montgomery, O'Leary and Barker[45] had elaborated this concept under the term nodular vasculitis. In spite of the denial by some that it is not an entity[2] distinct from erythema induratum, excellent studies in support of the identity of nodular vasculitis have appeared. In 1949, Woodburne and Philpott[78] reported 6 cases, Vilanova[71] detailed the findings in 16 cases and Irgang[29] observed 2 examples in Negro cases. In addition, case reports in various society proceedings have recorded a number of such patients, for example[47]. The term nodular vasculitis was introduced by Montgomery, O'Leary and Barker[45] to identify a variant of erythema induratum in which no evidence of tuberculosis could be found. This idea originated with Audry[3] who demonstrated such a patient in 1898. Because in the same year, Thibierge[69] presented a series of cases in younger women in whom tubercle bacilli were found, considerable discussion arose notably among the British dermatologists. Galloway[21] pointed out that there were indeed two types of erythema induratum, one nodular tuberculosis, the other entirely circulatory. Both he and Savil[60] stressed the "solid edema," the mottled appearance and the "vascular paralysis." No evidence of tuberculosis could be found in the cases presented.

Whitfield[75] was also an early investigator of these conditions. He called attention to the onset of the disorder after the age of thirty, with more pain and more rapid healing of individual lesions in the nontuberculous type. There was an associated rheumatism in many cases as well as dyspepsia, palpitation or other functional disease of the nervous system. Two of his patients gave evidence of various other dysf[illegible]ons.

In discussing the histopathology, Whitfield points out the atrophy of fat, which he claims is not qualitatively different from that seen in simple starvation and the cellular infiltration which is responsible for the "solid edema" of Galloway. He insists that these changes are nonspecific, that they closely resemble the changes of tuberculosis and must not be relied

upon to provide a diagnosis. Only bacteriological examination can be depended on to do this. His belief was that in the nontuberculous form an etiologic agent would be found whose role corresponded to that of the tubercle bacillus in typical Bazin's disease. Later Galloway called attention to the vascular changes consisting of infiltration and endothelial proliferation which he was able to distinguish from those occurring in syphilis.

During the following years terminology was discussed and simplified, dropping such terms as dermatitis necrotisans and periphlebitis nodularis necrotisans, and the search for etiologic agents continued. As stated above, Sulzberger[64] commented that "all typical cases (of Bazin's disease) in which extensive thorough and proper examination has been made have been shown to be of undiscoverable cause, or have eventually been shown to be of tuberculous origin." Proper investigation would consist of epidemiologic and immunologic studies, and biopsies of lesions in various stages of development. Each tissue removed should be subjected to histologic and bacteriologic study with portions of each injected into as many as ten guinea pigs.

In redirecting interest to nodular vasculitis, Montgomery *et al.* adopted four points as evidence of tuberculous origin of erythema induratum:

1. Positive reaction of guinea pigs to inoculation.

2. The presence of accepted histopathologic changes of tuberculosis, including the presence of typical tubercles, with or without demonstration of tubercle bacilli, and not merely collections of epithelioid cells and foreign body giant cells or the presence of caseation necrosis without the formation of tubercles.

3. Contributory evidence such as proof of active tuberculosis elsewhere in the body or a history of previously recognized tuberculosis, positive roentgenologic findings or strongly positive tuberculin reactions.

4. The clinical appearance and typical course of accepted types of cutaneous tuberculosis.

Montgomery and his co-workers use the term nodular vasculitis to refer to relatively chronic persistent or recurrent nodular lesions of nontuberculous origin occurring chiefly on the legs below the knees. Occasionally they ulcerate. They are found especially in women past the age of 30, but sometimes in younger women and infrequently in men and boys. The clinical manifestations resemble those of erythema induratum so closely that observation over a period of several years has, in some cases, been necessary finally to exclude the latter disease.

In a later discussion O'Leary[48] pointed out that the lesions of nodular vasculitis are not as large nor as indurated as those of erythema induratum. They recur in crops but there may be periods of several months during which no lesions appear. Hypertension, while common in these patients, is usually of moderate grade. In the same discussion Montgomery said there is no deep ulceration or true tubercle formation in nodular vasculitis and that the lesions are more persistent than those of erythema nodosum.

Irgang[29], in his report, showed nodular vasculitis developing in two women with definitely elevated blood pressure. He remarks that the hypertension may in this age group, be merely a concomitant finding, but the presence of vascular changes in the involved areas suggest the possibility that cutaneous lesions may be a local manifestation of generalized disease.

Woodburne and Philpott[78] presented 6 patients with nodular vasculitis. Of these, two appeared in association with

infected teeth or tonsils, one with phlebitis migrans, one with the phlebitis accompanying colitis and anal fistula and two were not accompanied by any apparent causal association. Vilanova's[71] 16 cases of nodular vasculitis may be taken as presenting the typical clinical picture of this type of nodose lesions of the legs. The patients were women who varied in age from 15 years to 60 years. The disease had persisted in his patients for from 1 to more than 5 years. Among the 16 patients the lesions were located chiefly on the lower extremities, usually bilaterally, mostly on the posterior lateral aspects of the legs. Pain or tenderness was a variable finding. In size, the lesions varied from a pea to confluent plaques. They also varied in number in individual patients (from 1 to many). They were normal skin colored to reddish violet, rose to vivid red. They evolved in about from 1 to 3 months. The lesions adhered to the skin and some of them had become ulcerated (7 of 16). In some cases fistulae resulted. The nonulcerative nodules were resolved within 15 to 30 days. In some cases there was some lymphatic or other evidence of tuberculosis. In 9 cases foci of infection in tonsils or teeth were uncovered. The sedimentation rate was increased in a number of cases. Tuberculin tests (O. T.) gave intense positive reactions in all patients, with extreme local, even necrotic, phenomena in some. No general nor focal reactions were observed. Intracutaneous tests with certain bacteria, nonspecific protein and leishmanin yielded positive reactions in all cases, but of variable intensity and less than that of the tuberculin reaction. These reactions may represent paraallergic reactivity in patients even with tuberculous terrain. The nodular vasculitis was associated in 6 cases with erythema pernio, with alopecia areata and eczema, asthenia and some loss of weight and a minor chorea. In 2 cases it was accompanied by cough and rheumatism. The 2 common features in all 16 cases were hypersensitivity to tuberculin and to a lesser degree to other bacteriologic and protein antigens and secondly the preferential localization on the lower extremities, a site frequently affected by erythema pernio. These 2 facts led Vilanova[71] to suspect that the alterations in the vascular periphery physiopathology can play a role in this condition. An additional clinical concomitant of nodular vasculitis is hypertension[29,50].

Histopathology of the Nodular Lesions of the Legs. The histopathological picture of the nodular lesions of the legs centers about 4 cardinal features: vasculitis, cellular infiltration, granuloma formation and fibrosis. These will vary in type, location and severity not only in the different conditions but in the different stages of one condition and to be more confusing, from case to case in any given stage or disease. It should be noted at the outset that caseation necrosis and giant cell formation are not to be regarded as cardinal features, although their presence or absence may favor one diagnosis or another. In addition, one or more of the four features mentioned may be entirely lacking.

The following table by Winer[77] summarizes the microscopic picture in nodose lesions of the lower extremities:

ERYTHEMA NODOSUM. Tachau[67] placed the primary trouble in erythema nodosum in the subcutaneous fat, where according to Winer[77] the first change is vascular. He notes edematous dissociation of the layers of the wall of larger vessels, especially arteries, and hyalinization in the smaller ones. Fibrinoid degeneration has been noted by Bergstrand[10] to involve only a segment of the vascular wall in the man-

TABLE 1.—MICROSCOPIC PICTURE IN NODOSE LESIONS OF THE LOWER EXTREMITIES

	Erythema Nodosum	*Erythema Induratum*	*Nodular Febrile Nonsuppurative Panniculitis*	*Periarteritis Nodosa*
Epidermis and upper part of cutis	Not involved	May have areas of necrosis and ulceration	Mild involvement	Hemorrhage and necrosis
Lower part of cutis	1. Mild polymorphonuclear cell infiltrate 2. Mild edema 3. No necrosis	1. Diffuse lymphocyte infiltrate 2. Caseation necrosis	1. Mild perivascular lymphocyte infiltrate and edema 2. Anuclear caseation necrosis	1. Diffuse lymphocyte infiltrate 2. Perivascular necrosis
Subcutaneous interlobular septums	1. Arteritis, acute *a*) Dissociation type *b*) Nonobstructed 2. Nests of polymorphonuclear cells 3. Eosinophilic cells 4. Reticuloendothelial nodules 5. Perilobular giant cells 6. Fibrillary degeneration	1. Obliterative organized thrombotic vasculitis 2. Chronic lobulated granuloma *a*) Epitheloid *b*) Giant cells *c*) Lymphocyte border *d*) Fibroblastic proliferation 3. Caseation necrosis	1. Arteritis, edematous type 2. Syncytial edema 3. Sparse cell infiltrate	1. Arterial obstruction *a*) Thrombosis *b*) Sectoral and segmental involvement *c*) Intimal proliferation *d*) Partial loss of lamina elastica interna *e*) Media dissociated by edema and cell invasion *f*) Pleomorphous cell infiltrate 2. Collagen necrosis
Fat lobule	1. Normal size 2. Intact: no necrosis	1. Shrunken 2. Replacement atrophy nuclear increase ("Wucheratrophie") 3. Caseation necrosis	1. Entire lobule involved 2. No nuclear increase 3. Serous necrosis of fat cells (lobule looks like sebaceous gland) 4. Phagocytic histiocytes and giant cells	1. Liquefaction of fat 2. Caseation necrosis of collagen
Terminal fibrosis	None No scar	Diffuse cutis and subcutis fibrosis Depressed scar	Diffuse cutis and subcutis fibrosis Depressed scar	Perivascular fibrosis Calcinosis Depressed scar

ner of periarteritis nodosa. Lever[35] comments on the endothelial proliferation. Vascular changes remain mild, however, and thrombosis, necrosis and occlusion do not occur, despite occasional marked perivascular hemorrhage.

The cellular infiltrate consists of polymorphonuclear leukocytes, some of which may be degenerated and occasional eosinophils and lymphocytes. These center in and about the vessel walls and in the interlobular septa of the subcutaneous fat, where they push themselves between the fat cells at the periphery of the lobule. As the lesions age lymphocytes become more prominent, and the polymorphonuclear leukocytes tend to disappear.

The granuloma formation appears to be of two types. Miescher[44] and Winer have found nodules of fibroblasts radiating from the vicinity of the small vessels. These could not be distinguished from the nodules described by Ophuls[52], Zeek[80] and Bergstrand[10] in periarteritis nodosa. Despite these changes the development of fibrosis is minimal.

A second type of granuloma occurring in erythema nodosum is that ordinarily associated with the presence of foreign bodies. This often occurs late in the course of the disease, and is secondary to the necrosis and phagocytosis of fat particles. When this occurs there may be a secondary thrombophlebitis of the small veins.

All of the changes described are completely reversible and resolve with no residual scar or depression of the overlying epidermis.

ERYTHEMA INDURATUM (BAZIN). Erthema induratum and papulonecrotic tuberculid, a smaller and more superficial lesion not infrequently occur together. Both have been classified by Montgomery as tuberculosis cutis indurata, and are by definition hematogenous tuberculous vasculitides. They start with endothelial proliferation occasionally associated with fibrinoid degeneration of the vessel wall similar to that seen in periarteritis nodosa. The lumen is obstructed by endothelial cells and thrombus formation. From the wall small epithelioid tubercles develop and enlarge. Soon the central mass consists of packed endothelial cells, thrombus, smooth muscle and epithelioid cells and infiltrating lymphocytes, and the vessel wall loses its identity as such. Elastic tissue stain will however demonstrate the remnants of the elastic membrane. From the first there is a considerable lymphocytic infiltration in and around the area. Caseation necrosis sets in and the tubercle is formed. Giant cells of the Langhans type are usually found.

The larger and deeper lesions of erythema induratum soon involve the subcutaneous fatty tissue. The individual fat cells are separated by infiltrating lymphocytes and proliferating histiocytes, the latter being phagocytic. These by taking up the liberated particles of necrosed fat assume a superficial resemblance to the foam cells of xanthoma. Others unite to form giant cells. Thus we have on the periphery of a truly tuberculous granuloma a foreign body reaction which may closely simulate the original process. A form of caseation necrosis may ensue making the differentiation even more difficult.

Because of the vascular occlusion necrosis may extend to the surface and ulceration result.

Fibrosis developing as the process subsides replaces the destroyed fat lobule, and the resulting scar is depressed below the skin surface in many instances.

At times, as a result of necrosis of the fat or extensive fibrosis secondary to the vasculitis, vascular changes may

predominate to the exclusion of any formation of tubercles.

ERYTHROCYANOSIS: PERNIO: NODULAR VASCULITIS. The histopathological picture of erythrocyanosis as outlined by Telford consists of:

1. A thickening of the swollen arterioles, all three coats being affected, and a dense perivascular infiltration of lymphocytes.

2. The presence in some cases of numerous giant cells swollen by fat.

3. Fat necrosis in various stages of resorption. This is the dominant picture.

4. The formation of fibroblasts and granulation tissue in the later stages. This is evident in the necrosed area.

In fact, so complete is the identity, that unlabeled sections of tissue affected with erythrocyanosis, Bazin's disease, anterior poliomyelitis and fat necrosis of the breast cannot be sorted into their respective groups.

An identical picture is presented by McGovern and Wright[40] in cases of pernio. In addition, they mention necrosis of fat which is suggestive of but is not true caseation.

Of nodular vasculitis Montgomery, O'Leary and Barker[45] say: Histopathologically the nodules showed evidence of vasculitis with varying degrees of thickening and obliterative changes in both veins and arteries, and a varying degree of fibrosis of subcutaneous tissue with collection of foreign body giant cells and atrophy of the fat, but without definite tubercle formation.

In Irgang's cases of nodular vasculitis the changes were somewhat less severe. They were located in the subcutis and consisted of thickening of the vessel walls and narrowing of the lumen. There was slight swelling and occasional proliferation of the intima. Replacement of fat by fibrous tissue was prominent, and there were a few small areas of fat necrosis, although giant cells were absent. Small dense infiltrates of lymphocytes were located about some of the smaller vessels.

These two cases seem to fall midway between those reported by Montgomery *et al.* and some of the changes reported by Hines and the group working with him. Indeed, considering the difference of age and blood pressure of the patient, one of Irgang's photographs might be superimposed upon one shown by Farber and Winer[19], and Hines, Montgomery and Craig[28]. These authors studied the changes in the normal skin of hypertensive patients. They found a decrease in the wall to lumen ratio of arterioles as compared to normal persons. Other changes consisted of hyperplasia of the muscularis and thickening of the internal elastic membrane. Endothelial hyperplasia and proliferation were met with as well.

Such changes were also found, associated with perivascular infiltrates of lymphocytes and hyalinization of the media, in the ischemic ulcers occurring on the legs of persons with hypertension. They were similar to those reported to occur in the pectoral muscle in hypertension.

Finally, Kulwin and Hines[34] in a study of vascular changes of the skin associated with long standing venous stasis (varicosities or thrombophlebitis) found the predominant changes again in the arterioles. The intima was thickened and proliferated and the muscular layer hypertrophic. Fragmentation of the internal elastic membrane was noted in some cases. Changes occurred in the veins as well but were less severe and much less frequent.

All of these changes were found to be unrelated to concomitant hypertension, dermatitis or ulceration. In three cases where arteriolar occlusion appeared, the section had been taken from the vicinity of an ulcer.

The causal relationship of the stasis

to the arteriolar change was not determined.

PHLEBITIS MIGRANS. Several types of phlebitis may cause confusion in making a diagnosis of nodular vascular diseases. One is that which occurs in about 20% of cases of thromboangiitis obliterans. This, while resembling other forms, is pathologically identical with thromboangiitis and is accompanied by the clinical manifestations of that disease.

In some cases of far advanced tuberculosis, painful inflammatory reactions occur about superficial veins. Fusiform swellings develop, involving the skin. Thrombosis however does not occur in cases associated with tuberculosis.

There is another variety of phlebitis usually in a larger superficial vein, associated often with heavy smoking and occurring primarily in men. Constitutional symptoms are mild, and ulceration does not occur. The lesions are tender and somewhat fusiform.

They recur in crops, each lasting one or two weeks and will, by involving successive segments travel the entire length of the vein. Histologically the vessel wall is infiltrated with polymorphonuclear leukocytes and the layers separated by edema. Thrombosis may or may not occur and the perivascular tissues are not involved. There may be some fibrosis of the vessel wall.

Etiologic Considerations. Since the tubercle bacillus is intimately associated with some of the pathological processes under discussion we are justified in taking a closer look at its role in etiology. Sabin and Doan[59] have shown that a protein fraction isolated therefrom incites a "clasmatocytic response" with toxicity, fever and multiple hemorrhage. The clasmatocyte, they point out is a phagocytic cell (histiocyte?) capable of rapidly fragmenting the tubercle bacillus. A phosphatide fraction, on the other hand, stimulated formation of epithelioid cells and Langhans type giant cells. Exactly what this means in terms of pathogenesis we are not prepared to say. The following facts may, however, be pertinent.

Erythema nodosum has been shown to be a phenomenon of developing "allergy" to any of several sensitizing substances, one of which is the tubercle bacillus. It is a transitory phenomenon whose appearance is often associated with the development of a positive skin test and a favorable prognosis. Bergstrand[10] considers, however, that it may be an anaphylactoid reaction similar to the Arthus phenomenon and more clearly related to periarteritis nodosa.

One point has been demonstrated definitively, that is, whatever the cause or the mechanism of development, one lesion is indistinguishable from another due to a different cause.

Little has been said about the role of tuberculosis in periarteritis nodosa. Ruiter[58] noted frequent occurrence of tuberculosis in his cases of arteriolitis allergica, and he quotes Miescher as finding an unusually high incidence of tuberculin sensitivity in cases of periarteritis nodosa. The case reported by Gilrane and Cherry[22] is of interest in that glandular tuberculosis proven bacteriologically, developed in a patient receiving cortisone for nodular lesions of the legs diagnosed as periarteritis nodosa. Both the glandular tuberculosis and the nodular lesions of the legs healed when the patient was given streptomycin and PABA (para-aminobenzoic acid). The case was further complicated because one member of a panel of pathologists, who originally made the diagnosis of periarteritis nodosa, disagreed, preferring instead a diagnosis of sarcoidosis.

Knap[32] surveyed 63 cases of erythema induratum that had been in a 12-

year period. Diagnosis was based on clinical history, Mantoux test, biopsy, Roentgen examinations and history. The results of his survey are compared with the results given by Montgomery *et al.* in 72 cases.

	Montgomery et al.	*Knap*
Mantoux Positive	84%	100%
Papulo necrotic tuberculid (concomitant)	5%	20%
Typical biopsy	70	70%
Tubercle bacilli seen in tissue	3%	—
Guinea pig inoculation positive	2 of 10 cases in which done	
Positive chest Roentgen-ray	—	60%
Positive history of tuberculosis	—	78%

Of Knap's cases none had died of tuberculosis in an average observation period of 6 years. Three had developed severe lesions in the lungs and five more developed benign inactive pulmonary changes.

Other factors than the tubercle bacillus have received attention in the development of erythema induratum. Bazin believed that the suppression of menstruation was significant in some cases. The effect of "chilblain" has been commented upon.

Knap and Danbolt[32] have investigated the ovarian function in women suffering from erythema induratum and discovered a deficient excretion of estrogen in a substantial per cent. Gonadotropins and androgens were not affected.

After citing the vasodilating effect found by Racine and detoxifying effect on various types of anesthetic agents described by Stortebecker, Knap postulates that in a tuberculin positive individual tuberculosis toxins and antibodies may circulate in the blood. It is reasonable to believe that both these groups may be able to sensitize other organs than those which harbor tubercle bacilli. In some individuals especially those with erythrocyanosis there will be the possibility of toxic effect. This possibility will be greater if the individual has a deficient estrogen production. In the first place the protective antitoxic effects of these will be lacking, and in the second place the loss of the vasodilatory effect will cause or intensify an erythrocyanosis crurum. Clinical experience shows that this is a frequent coincidence and may be one of the conditions necessary for the development of erythema induratum. The ovarian dysfunction represented by reduced estrogens in patients with erythema induratum must therefore be regarded as a significant etiological factor in addition to the mycobacterium tuberculosis.

Differential Diagnosis of Nodular Vasculitis. Erythema induratum, as might be expected, is not easily distinguishable from nodular vasculitis. The age of incidence is cited as a point of differentiation, and so it is. Yet Knap found 17 of 63 cases of erythema induratum over the age of 30 and of Woodburne and Philpott's 6 cases of nodular vasculitis 2 were under 20.

Ulceration, another point of differentiation, was absent in 10 of Knap's cases. He cites 209 other cases, reported by various authors, in whom ulceration was absent in 112. This ulceration is likely to be deep, with circular outline and hypertrophic overhanging margins. There has been no tendency for the cases reported as nodular vasculitis to heal in summer and recur in cold weather, a feature common in erythema induratum and erythrocyanosis and pernio.

ERYTHROCYANOSIS AND PERNIO. Telford under the name erythrocyanosis discusses a mottled discoloration of the legs which blanches on pressure and is cool to the touch. It occurs on the calf, and just above the malleoli where it may form a firm elastic cuff. The fol-

licles are pigmented and from each protrudes a spiny hyperkeratosis. In the areas of discoloration nodules form and often proceed to ulceration. They are always worse in the winter and heal slowly in summer. The patient is almost always a woman of stout build and florid complexion.

The process resembles closely that seen in the limb paralyzed by poliomyelitis where absence of muscle action and reduced fascial tension permit pooling of the blood in the venous channels.

Telford considered these changes to be identical with those occurring in erythema induratum. Since in the cases he investigated no evidence of tuberculosis could be found, he believed erythrocyanosis and erythema induratum of Bazin to be the same disease.

McGovern and Wright[40] describe as pernio, a chronic recurrent nodulo-ulcerative condition which they believe similar to erythrocyanosis. This also occurs in women of stout build. It is manifested by a reddened area, somewhat indurated and painful. The color becomes violaceous and the area fluctuant. Finally, it forms a shallow ulcer, discharges a serosanguinous exudate and becomes less painful. Healing is slow, with the formation of a pigmented scar in which the next winter's crop of lesions develop. Throughout the years this leads to a sclerodermatous-like hardening of the skin which can be quite disabling.

These authors cite Mordelli as differentiating between pernio and erythema induratum on the basis of seasonal incidence which, in his opinion, the latter does not show. He believes, however, that the two may co-exist, but that tuberculosis is always a complication and not a cause of perniosis.

HYPERTENSIVE ULCERS. These lesions, described by Hines and Farber[27] appeared on the legs of persons whose chief or only other physical abnormality was hypertension. In some cases the first change consisted of a red painful plaque in the skin, on which an ulcer developed. In others the ulcer developed on normal skin. It appeared as a purpuric spot, followed by vesiculation, ecchymosis, necrosis and ulceration. The ulcers were described as being ischemic in character, such as occur in vascular occlusion, pernio, livedo reticularis or senile changes. Healing was slow.

No evidence of stasis, livedo varicosities, or dermatitis was noted, and the lesions most closely resembled self-induced ulcers or ulcers due to drugs or blood dyscrasias.

PERIARTERITIS NODOSA. For several reasons it is difficult to evaluate the precise role of periarteritis nodosa in these cutaneous conditions. First, the skin manifestations of periarteritis nodosa vary, and the reasons for this variation are not readily apparent. Second, the causes of periarteritis are several and not readily differentiated from the causes of some of the nodular skin lesions. Third, the meaning of the term periarteritis nodosa has only recently been clarified. Attempts have been made to improve the terminology by adding such terms as periarteritis to indicate the involvement of all the layers of the wall, and polyarteritis to indicate the involvement of arteries in many locations. The recent work of Zeek and her collaborators appears to have merit. Like others she has found it necessary to introduce new terms. She would refer to the group as necrotizing angiitis to indicate essential pathological histology. Under this she sees 5 groups.

1. Sensitization angiitis (serum and sulfonamides).

2. Allergic angiitis associated with asthma or Loeffler's syndrome.

3. Periarteritis nodosa: the classical form of Rokitansky, Keissmane and Meier.

4. Rheumatic form, associated with fulminating rheumatic fever.

5. Temporal arteritis.

On one side of this classification it is possible to "hang" such conditions as erythema nodosum, a benign angiitis occurring in the course of "sensitization" to other infections or drugs. In a like manner one may on the other side hang lupus erythematosus disseminatus with its polyserositis, angiitis, its similarity to the rheumatic diseases and its serious import. Other parallelisms will be seen.

With their technique for producing the periarteritis nodosa picture in rats Zeek, Smith and Weeter[80] have established a basis for the comparison of clinical and necropsy material in the various types of angiitides.

Many of the points they discuss have no direct bearing on cutaneous manifestations but one, the simultaneous involvement of arteries and veins is pertinent, and has been mentioned frequently. Zeek[79] and the others have shown that in "periarteritis nodosa" the primary involvement is always at the site of bifurcation of a medium-sized artery. The vein is involved later and by peripheral extension. In the angiitis of sensitization both arteries and veins, of smaller caliber show simultaneous involvement. This is a very easily overlooked difference which may require many serial sections to demonstrate. It may help, however, to explain some of the varied cutaneous manifestations of periartitis nodosa.

Illustrations in Miescher's[14] article show one case resembling a papulonecrotic tuberculid. Another might be mistaken for syphilis. Slinger and Starck show a case demonstrating a livedo racemosa configuration. Also, according to Ruiter[58], Carol and Praaken[12], Goldschlag[23], Crosti[14] all have cases showing livedo racemosa. The case described by Ketron and Bernstein had this configuration. Until the physiology of livedo reticularis is better understood we shall probably not be in a position to account for this distribution. Ketron and Bernstein believed but could not prove, that the chalky areas in their patient corresponded to the central arterial cone.

Another view opened up by Zeek's classification is the distribution of skin lesions, as well as their type, among the various angiitides. Logue and Mullins[38] report 11 cases in which 7 had skin manifestations. This is well above the average usually given (25% Ketron and Bernstein; Nodule 23%, others exclusive of purpura 4%, Harris Lynch and O'Hare[25]). Of these 7, one had temporal arteritis, hypertension and characteristic excoriated papules of the abdomen. One had a pulsating nodule, two had painless firm nodules, two had purpura, and one had early in the disease a pruritic vesicular rash, followed later, not necessarily in the same spots, by pigmentation with central scarring. This patient was the only one of the 7 showing skin lesions who did not have hypertension.

Churg and Strauss[13] in 13 cases associated with severe asthma found purpura in 8 and subcutaneous nodules in 7. In 3 of these there were granulomatous nodules of degenerated collagen with radially arranged macrophages and giant cells. In 2, the nodules consisted of several foci of fibrinoid degeneration of collagen, and in one there was homogenization of collagen as seen in rheumatoid arthritis. All lesions were paravascular and there were no vessel changes in the skin. Internal vessels showed the necrotizing angiitis.

Similar granulomata have been reported in the nose, Weinberg[74]; Symmers and Litchfield[66]. These latter lesions, as well as those of Churg and Strauss are of the paravascular type.

TABLE 2.—DIFFERENTIAL DIAGNOSIS OF NODULAR VASCULAR DISEASES OF THE LEGS

Disease	*Clinical Features*	*Vascular Changes*	*Histologic Changes*	*Etiology (Remarks)*
Nodular Vasculitis	Concept of Galloway and Whitfield 1899–1913. Painful nodose lesions calves of 30-40-year-old women, rarely younger. Clear on elevation and rest. Ulceration rare, recurrent, more painful and of shorter duration than erythema induratum.	History of phlebitis	Evidence of vasculitis. Varying degrees of thickening and obliterative changes in veins and arteries. Varying degree of fibrosis. Collections of foreign body giant cells. Fat atrophy. No tubercle formation.	Non-tuberculous (non-tuberculous erythema induratum?)
Erythema Induratum (Bazin)	Ulcerative or nodose calves of legs of girls during adolescence. May affect all ages and both sexes. May be associated tuberculosis elsewhere. Tuberculin test positive. Guinea pig inoculation may be positive or negative.	Varying degrees of vasculitis in one-third of cases. Varices present.	May be nonspecific (granuloma). Three types of changes: (*a*) formation of typical tubercles with caseation in center of tubercles. (*b*) Severe degree of necrosis of fat, acute and chronic, varying degrees of vasculitis and foreign body giant cell reaction. (*c*) Same as (*b*) but with fibrosis rather than necrosis of fat predominating.	Tuberculous
Erythrocyanosis (and Pernio)	Reddish blue discoloration of skin on lower half of legs of girls and women. May be associated with nodules, resolving or ulcerating. Women are florid and have large legs. Recurrent attacks in winter, disappear in summer. Ulcers in pernio more superficial than in erythema induratum.	Vasculitis present	Foreign body giant cells, obliterative vascular changes, thickening of all coats of arteries, fat necrosis, granulation tissue.	Non-tuberculous
Erythema Nodosum	Acute; associated fever, malaise, arthralgia, superficial tender nodules and plaques on legs usually last few days or weeks. No ulceration. Leave discoloration.	Secondary thrombophlebitis may supervene.	Infiltration about vascular network in upper and middle portions of cutis. Lymphocytes and polymorphonuclears. Vessel walls edematous. Inflammatory hemorrhagic infiltration of walls. Acute inflammation with fat atrophy may be present.	Streptococcic. Various: *e. g.*, syphilis, coccidioides, drugs; (bromides, sulfonamides).

TABLE 2.—DIFFERENTIAL DIAGNOSIS OF NODULAR VASCULAR DISEASE OF THE LEGS (*Cont.*)

Disease	*Clinical Features*	*Vascular Changes*	*Histologic Changes*	*Etiology (Remarks)*
Panniculitis (Weber-Christian Disease)	Febrile bouts. Large subcutaneous plaques and nodules, especially on trunk and thighs. Subsequent subcutaneous atrophy with depressions of skin pore. Women chiefly affected.	Little evidence of vasculitis.	Edema and necrosis of fat with phagocytic cells of various types which engulf the fat inflammatory reaction: polymorphonuclears and lymphocytes. Little vasculitis or fibroblastic proliferation but may be even thrombosis.	Non-tuberculous
Recurrent Idiopathic Thrombophlebitis	May occur in conjunction with most of above states; may be secondary to other pathologic processes. May be *primary*. This affects *men* chiefly. Disease of small or medium-sized veins. Multiple discrete tender nodules. Last 7–18 days. Crops linear to palpation. Smaller than erythema nodosum and induratum. Mild constitutional symptoms.		Occlusion of lumen of vein by cellular thrombosis. Severe infiltration of wall of vein. Fibrosis of wall. Very little or no perivenous inflammation. No fat necrosis nor fibrosis.	
Indurated Cellulitis and Ulceration Secondary to Chronic Venous Stasis	Preceding chronic venous stasis after iliofemoral thrombophlebitis or varicose veins. Subacute or chronic inflammatory reaction lower third of the leg producing tender painful plaque-like induration of the skin and subcutaneous tissue. Ulceration may follow secondary infection.	Varicose veins of long standing	Pathology of thrombosed veins	Non-tuberculous stasis

In attempting to fix the exact site of the primary disturbance certain difficulties are met. One is that in the vessels above the subcutaneous plexus the anatomical difference between arteries and veins is very slight. These slight changes may be lost in the general tissue reaction. Another is that since arteries and veins lie so close together any considerable reaction is likely to involve both, making localization of the initial process impossible.

About most of the conditions of interest in this discussion there is some doubt. In nodular vasculitis, erythema induratum and sarcoid, arteries and veins, seem involved to about the same extent. Telford[68] attributes erythrocyanosis to arteritis and McGovern and Wright localize pernio in the veins.

Winer believes the primary change in erythema nodosum to be in arteries, but Lever[35], Löfgren and Wahlgren[37] place it in the veins.

The changes associated with hypertension with or without ulceration, syphilis and with chronic venous stasis are localized to the arteries and arterioles.

In studies of disseminated lupus erythematosus, Lowman and Slocumb[39] found the primary vascular lesion in muscles and nerves to be venous predominantly, but in the skin the changes are arterial (Ehrich).

In periarteritis nodosa the location of the primary process is one of the criteria used by Zeek *et al.* in making their subclassification. In the "true" periarteritis nodosa the lesions are always arterial but involve the veins by peripheral spread. In the sensitization angiitis, the lesions begin simultaneously in both arteries and veins.

SARCOID. Sarcoid should be mentioned, perhaps, only as a point of differential diagnosis. It was at one time thought by some that the subcutaneous sarcoid of Darier-Roussy was identical with erythema induratum. However, it is the feeling of most pathologists at present that the sarcoids of Boeck and Darier-Roussy differ only in location and that they have the same cause, or multiplicity of causes.

The salient features of Montgomery, O'Leary and Barker's paper[45] will serve as a useful summary of this discussion.

A group of diseases with many features in common includes: nodular vasculitis; erythema induratum; erythrocyanosis and pernio; erythema nodosum; panniculitis; recurrent idiopathic thrombophlebitis and indurated cellulitis and ulceration secondary to chronic venous stasis.

The differential problem is difficult. Histologic changes may be typical of tuberculosis and yet not be of that causation and *vice versa* since tubercle bacillus may produce a banal nonspecific inflammatory reaction in some cases. When histopathologic changes are presumptive, procedures to establish tuberculosis must be performed. Prolonged observation may reveal tuberculosis, which may be absent at the first contact with the patient. Removal of *many* specimens in different stages of development for histologic study is suggested. Portions of each should be used to inoculate as many as ten guinea pigs. Table 2 on pages 483 and 484 is a good summary of this problem.

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BOOK REVIEWS AND NOTICES

ANTISEPTICS, DISINFECTANTS, FUNGICIDES, AND CHEMICAL AND PHYSICAL STERILIZATION. Edited by GEORGE F. REDDISH, PH.D. SC.D. (HON.), St. Louis College of Pharmacy and Allied Sciences. Pp. 841; 71 ills. Philadelphia: Lea & Febiger, 1954. Price, $15.00.

FOR many years the standard reference book in the subject of disinfection was McCulloch's "Disinfection and Sterilization." As time went on, however, the last (1945) edition gradually became outdated and need arose for a worthy successor. Dr. Reddish and his contributors have more than adequately filled this need. The new volume is more comprehensive in detail and broader in scope than its predecessor. Because each of the 30 authors is writing about his field of special interest, most of the 35 chapters reflect a complete command of the subject.

One entire section is devoted to detailed descriptions and evaluations of methods. Not only are all of the major classes of disinfectants handled as separate entities but they also turn up repeatedly under such headings as Antiseptics, Fungicides, Viricidal Agents and so forth. Sterilization by physical means is also included, and the chapters on the use of heat and gases deserve special commendation.

Because it is a dependable source of information in the broad field of disinfection, exclusive of chemotherapeutic drugs, this book will undoubtedly enjoy the wide usage it deserves. And of particular value to physicians and nurses are the chapters on surgical antisepsis and the disinfection of surgical instruments. E. S.

THE MEANING OF SOCIAL MEDICINE. By IAGO GALDSTON, M.D., Secretary, Medical Information Bureau, New York Academy of Medicine. Pp. 137. A Commonwealth Fund Book. Cambridge, Mass.: Harvard University Press, 1954. Price, $2.75.

THIS highly provocative little book raises many questions and provides few if any final answers. Nevertheless it should be read by every physician who recognizes the jeopardy in which medicine stands today in spite of its amazing accomplishments in immunology, etiologic diagnosis, definitive therapy, and so forth. Five years as administrative officer of the "Committee on Medicine and the Changing Order" of the New York Academy of Medicine, provided the author with much of the inspiration and background that resulted in this book.

The title of Chapter One is "What is Social Medicine?" Apparently there is no satisfactory answer. It is not socialized medicine." Perhaps it is something like a combination of curative medicine plus preventive medicine and public health, to which a new orientation has been given by insisting that not just the patient's disease, but the *whole patient in and with his environment* must be the object of diagnosis and treatment. About the only presently existing example of a discipline resembling "social medicine" is pediatrics with emphasis on well-baby clinics.

Many readers will agree that parts of this work are open to serious question. For example, after insisting that "despite its triumphs and miracles, modern medicine has failed," the author indicates the broad lines that will have to be followed in order to turn that failure into success. It appears that future physicians will have to be supermen, with God-like knowledge and understanding! Indeed, they will have to be omnipotent rulers of mankind for they must straighten out the kinks in man's environment and rid him of ignorance and faulty ideas. It further appears that by conquering summer diarrhea, smallpox, diphtheria, pneumonia, and adding many years to man's life expectancy, "modern medicine has in a large measure converted mortality into morbidity." Those thus saved from early death "all too often have been saved only to longer endure the burdens of a variety of illnesses and to die at a later age." Does the author really believe that vaccination for smallpox, penicillin for pneumonia, and so forth should have been postponed until the degenerative diseases and death itself have been abolished?

The book's shortcomings are partly responsible for its provocative flavor. It is stimulating even if not inspiring. E. C.

DAS ELEKTRISCHE HERZBILD. By WILHELM ERNSTHAUSEN, DR.-ING., (F. KIENLE, M.D., Collaborator). Technical Institute Karlsruhe. Pp. 232; 227 ills. Munich: H. Rinn, 1953. Price, DM 48.00.

THE visual electrical concept of the heart is the graphic or numerical presentation of

those biological functions of the heart muscle that appear as electrical phenomena. Further progress in electrocardiography may be expected from a) widening of clinical experience in order to statistically improve diagnosis, and b) experimental construction of electrical heart models offering new theoretical and methodological possibilities.

Detailed description is given of the myocardium of the heart and its metabolism (movements of ions, transformation of energy), and aerobic and anaerobic influences are discussed. There follow electrophysiologic concepts such as resistance of membranes and tissues, electrical potentials, speed of excitation. The theory of the potential field about excited fibers and fiber groups is given. The applicability of vector operations on the electrical presentation of the function of the whole myocardium is questioned. There is an analysis of electrode systems, including vector cardiography and ventricular gradient. Interesting observations are reported on the T and S amplitude in its dependence upon the distance of the electrode axis from the fiber, and the relationship of spread of excitation in conduction system and heart muscle fiber, respectively.

Electrical heart function in a given patient, is depicted by many partial electrocardiograms, taken for the frontal plane, with an electrode distance of 3 cm. Such an illustration will show, for instance, 154 single E.C.G. complexes. A practical analysis was carried out on 1,719 patients, and there are 81 postmortem studies available. Particular attention is paid to ischemia, different types of infarctions, disturbances of excitation (WPW Syndrome, bundle branch block, myocardial disease, E.S.), diffuse damage (subdivided into inflammatory and metabolic disturbance), hypertrophy, insufficiency, and low voltage E.C.G.

An attempt is made to evaluate the functional status of the heart by a functional diagram that is based upon the gradation quotient and the refractory phase quotient. A chapter is devoted to a detailed mathematical-pictorial analysis by the Fourier method.

The illustrations and print are of superior quality. For a full understanding of the book, knowledge of biophysics and mathematics is a prerequisite. H. R.

NATURE AND STRUCTURE OF COLLAGEN. Edited By J. T. RANDALL, University of London. Pp. 269; 142 ills. New York: Academic Press, Inc., 1953. Price, $6.50.

THIS little book is a collection of "papers presented for a discussion convened by the Colloid and Biophysics Committee of the Faraday Society at King's College, London." It deals with the biological, chemical and physical properties of collagen, a structure the importance of which has become ever more keenly appreciated over the past decade. Since these papers were presented almost entirely by British workers, it is natural that much of the material is similar to that found in the papers of the Josiah Macy Conference on metabolic interrelations published in 1950.

Very few definitive answers are to be found in these papers; the exact chemical structure of collagen fibrils and reticulin is still unknown; whether the latter is transformed into the former is most uncertain, and the mechanism of the formation of collagen fibrils can only be presumed. (It is thought that it is a combination of cellular secretion and an extracellular environment.)

The material is presented in a very readable form and the discussions are pertinent. For the novice in this field it is probably easier reading than most of the American papers, largely, I suspect, because of the enviable talent of the English to get to the heart of the matter in the simplest and most lucid manner. E. A.

A MANUAL OF TROPICAL MEDICINE. By THOMAS T. MACKIE, M.D., COL., MC, AUS (RETIRED), Chairman, The American Foundation for Tropical Medicine; GEORGE W. HUNTER, III, PH.D., COL., MSC, USA, Chief, Section of Parasitology and Entomology, Fourth Army Area Medical Laboratory, Brooke Army Medical Center, Fort Sam Houston; and C. BROOKE WORTH, M.D., Field Staff Member, Division of Medicine and Public Health, The Rockefeller Foundation. 2nd ed. Pp. 907; 304 ills., 7 in color. Philadelphia: W. B. Saunders Co., 1954. Price, $12.00.

IT is a pleasure to welcome the second edition of this excellent work. Published during World War II, the first edition of this manual proved to be an exceedingly useful and practical presentation of the subject for the many military officers who found themselves confronted with the practice of tropical medicine. Since the war, there has been a continuation of research in this field and world conditions make it imperative for all physicians of this country to keep abreast of the newer knowledge. The authors and their

twenty-four collaborators are to be congratulated on the excellence of their product. The format is practical and the illustrations are excellent. The book should be in the hands of all medical students and young physicians.

R. K.

DISEASES OF THE SKIN. By OLIVER S. ORMSBY, M.D., Rush Professor of Dermatology Emeritus, University of Illinois, and HAMILTON MONTGOMERY, M.D., Professor of Dermatology and Syphilology, Mayo Foundation, University of Minnesota. 8th ed. Pp. 1503; 750 ills., 18 in color. Philadelphia: Lea & Febiger, 1954. Price, $22.00.

THE eighth edition of Diseases of the Skin by Ormsby and Montgomery continues the tradition of this work, which has served the medical profession since 1915. Because of the great volume of investigative work produced subsequent to the publication of the previous edition, it is surprising that so much has been added to this presentation without the necessity of making it a multi-volumed text. This has been accomplished by elimination of some obsolete material, condensation of other, and by the judicious use of smaller type here and there. If any criticism of this book is valid, it should be in the direction that it contains too much rather than too little. For the expert, however, this can hardly do any harm. The coverage, in addition to being voluminous, is also reasonably current. The content and presentation of this now standard volume has been enriched by the elevation to the status of contributors of men who have assisted in the past, DeLamater, Farber, Kierland and Lobitz, Jr.

H. B.

MEDICAL USES OF CORTISONE. INCLUDING HYDROCORTISONE AND CORTICOTROPIN. Edited by FRANCIS D. W. LUKENS, M.D., Professor of Medicine, University of Pennsylvania. Pp. 534; 52 ills. New York: The Blakiston Co., 1954. Price, $7.50.

FIVE years have passed since cortisone was added to the therapeutic armamentarium. Since then, a tremendous experience has been accumulated in the use of this and allied substances in many phases of clinical medicine. This publication meets the need for compiling and assessing these experiences and placing at the disposal of all physicians sound conclusions as to the value of these agents and advice as to their proper use. Doctor Lukens and his 29 contributors, all of them authorities in their respective fields, are to be congratulated upon the excellence of this work. There are chapters on the physiology of the adrenal cortex, the pharmacological aspects of adrenal cortical hormones and their effects in adrenal insufficiency and a wide range of clinical conditions in which they have been found useful. The book is highly recommended.

R. K.

BEYOND THE GERM THEORY. A SEMINAR. Edited by IAGO GALDSTON, M.D. Pp. 182; 18 ills. New York: New York Academy of Medicine, 1954. Price, $4.00.

"BEYOND THE GERM THEORY" is a collection of papers which illustrate factors, other than germs, which are etiologic in the ills of mankind. The papers deal with deprivation and stress and their interrelationship in disturbances of homeostasis. Such examples of physical deprivation as scurvy and beriberi are dealt with. The years of opposition by some of medicine's best brains to the idea that these diseases could be caused by a "lack of something," other than by some positive harmful influence, is presented and makes for interesting and humbling reading. One is reminded of present-day insistence that arteriosclerosis must be consequent to the ingestion of excess fat and that lung cancer is caused by too many cigarettes.

The tragic effects of emotional deprivation are strikingly illustrated in a well controlled experiment that contrasts the physical and psychological growth of 2 series of infants, one with mothers, the other without. A perusal of the differential notes of morbidity and mortality should convince the most adamant skeptic of the powerful force that is mother-love!

The book could profitably be read by all those working in the medical disciplines. It will receive special attention by those interested in comprehensive medicine and in the multifaceted approach to preventive and therapeutic medicine. The broad vistas of tomorrow's medicine are clearly portrayed.

W. S.

GESUNDHEIT DURCH KRANKHEIT. DAS RÄTSEL DER IMMUNITÄT. BY DR. HANS-JOACHIM FLECHTNER. Pp. 346; 46 ills. Düsseldorf: Econ-Verlag, 1954. Price, not given.

A presentation of the many aspects of immunity, pathogenesis of the body's methods of defense and the treatment and prophylaxis of infections, written in terms that laymen may understand it, nevertheless it presents an ac-

curate survey of the subject. The author has shown his skill as a writer and a scientist in presenting scientific material not only in this but in three other publications of similar type. Those who know German will find this work well worth reading. R. K.

ZUR PATHOGENESE DES ENDOGENEN EKZEMS. By DOZ. DR. MED. GÜNTER KORTING. Univ.-Hautklinik, Tübingen. Pp. 107; 23 ills. Stuttgart: Georg Thieme Verlag, 1954. Price, DM 13.80.

KORTING's monograph is one of a trilogy of presentations dealing with various types of eczema and emanating under the guidance of Gottron, based on the Breslau and Tübingen Clinics. Halter's essay deals with eczema vulgaris, Nikolowski discusses seborrheic eczema and the present, by Korting, is concerned with endogenous eczema.

Endogenous eczema is really what we call atopic dermatitis. The coverage of the subject is rather wide and includes clinical features, incidence, age and seasonal; constitutional aspects (for example, blood picture, intestinal tract and ocular manifestations); pathologic physiology and pathogenesis. The style of the German in which this volume is written makes for difficult reading but because of the many facets of the subject described and the bibliography, this work is useful for the allergist, internist, pediatrician and dermatologist.

H. B.

SCIENCE, MEDICINE AND HISTORY. Collected and edited by E. ASHWORTH UNDERWOOD. 2 volumes. Pp. 563 and 646; ills. 50 and 56, respectively. New York: Oxford University Press, 1954. Price, $45.00.

To honor Professor Charles Singer, long the dean of historians of science and medicine in Britain, where he has been associated with the Universities of Oxford and London, this work presents a remarkable group of essays on the evolution of scientific thought and medical practice. It has been edited by E. Ashworth Underwood, Director of the Wellcome Historical Medical Museum and of the Wellcome Historical Medical Library, London, and there are articles by 94 contributors: historians, scientists and physicians from all parts of the world, twenty-five of them from the United States. The essays cover a wide range of the whole field of the history of science and medicine, and many include important original research. The essays are all in English except five, four being in French, and one in Italian. They are arranged in eight books dealing respectively with The Ancient World, The Medieval World, The Renaissance, The New Philosophy, The Uncertain (17th) Century, The 18th Century, The 19th Century and Afterwards, and Some General Surveys. There are 106 half-tone plates as well as numerous figures in the text. There is also a bibliography of the published writings of Professor Singer. A complete index adds greatly to the practical value of the work. All historians will, of course, welcome this magnificent group of contributions, but all scientists, and especially physicians, will find here much to interest and instruct them, entertain and inspire them during many a pleasant hour. R. K.

HEART DISEASE AND INDUSTRY, WITH PARTICULAR REFERENCE TO WORKMEN'S COMPENSATION CASES. By MEYER TEXON, M.D. Pp. 324. New York: Grune & Stratton, 1954. Price, $7.50.

PHYSICIANS who have been called into court to testify as to the medicolegal aspects of cardiac disease have often wondered at the lack of an authoritative text that might serve as a guide for them in a field which is, to most of them, quite unfamiliar. Dr. Texon, who has had an extensive private and consultation practice in cardiology and who, for years, has participated in a popular short course for lawyers, has provided such a text from his wealth of experience. The greater part of the book is occupied by a discussion of 100 cases selected to illustrate typical problems. These are considered from the standpoint of the history, the findings on physical and laboratory examination, the medical opinion, the causal relationship of accident to disability, the prognosis, and the legal decision as to compensability. The book has been written both for the physician and for the lawyer, and includes a glossary of terms to help the latter. It is to be hoped that all physicians who have to deal with cardiac medicolegal problems will familiarize themselves with the contents of this valuable volume. It is to be hoped also that "with increasing attention paid by the medical profession to this entire problem, some of the apparent inconsistencies in the prevailing customs and laws concerning compensation for industrial accidents will be rectified" and that there may eventuate more uniform laws and legal interpretations in the various states of the Union.

T. D.